An-Najah National University
Faculty of Graduate Studies

THE IMMUNE AND METABOLIC IMPACTE OF TESTESTERONE ON MICE MODEL OF LIVER FIBROSIS

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This Thesis was defended successfully on 14/06/2023 and approved by:

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Dedication

To my parents ,husband, son, daughter, sister and brothers, who are the optimistic source in my life and to everyone who helped me to execute this work during my study and in my life.

Acknowledgements

I would like to thank my supervisor Dr. Johnny Amer for his time in helping me achieve my thesis and for Dr. Ahmad Salhab in helping me in the technical issues and methodology.

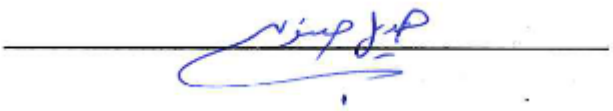
Declaration

I, the undersigned, declare that I submitted the thesis entitled:

THE IMMUNE AND METABOLIC IMPACTE OF TESTESTERONE ON MICE MODEL OF LIVER FIBROSIS

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: Hadeel Moneer Snober

Signature: 

Date: 14/06/2023

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THE IMMUNE AND METABOLIC IMPACTE OF TESTESTERONE ON MICE MODEL OF LIVER FIBROSIS

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Abstract

Background: Natural killer (NK) cells showed an anti-fibrotic effect; however, their function is thought to be impaired in advanced liver injury. The objective of this study was to evaluate the immune response and metabolic impact of testosterone on mice model of liver injury.

Methods: Carbon-tetrachloride (i.p injected) of acute (2 weeks) and chronic (4 weeks) models of male mice of liver injury was performed. Testosterone (4 mg/kg mouse body weight) was injected intraperitoneal injection following the first week of acute model of CCl_4 and following the second week of the chronic model of CCl_4. At the end of experiments, mice were sacrificed, and serum were collected for assessing liver enzymes of ALT, AST, inflammatory marker of IL-6, metabolic makers of C-peptide levels as well as for lipid and glucose profiles. Livers were harvested and used for histological assessments for inflammation and for fibrosis. Fibrosis profile from liver extracts; αSMA and Collagen III, The samples underwent assessment utilizing the real-time polymerase chain reaction (PCR) technique. Moreover, liver tissue-resident NK cells were isolated and evaluated for their activity through assessing INF-γ and IL-6 receptor by the ELISA and flow-cytometry respectively.

Results: The serum levels of ALT, AST, and IL-6, as well as metabolic evaluations of cholesterol, triglyceride, C-peptide, fasting blood sugar, and fibrotic profiles, exhibited a linear correlation with the progression of the disease. Histological characterization of the liver was worsened in the chronic model of liver injury. Testosterone-treated mice exhibit a significant reduction in collagen depositions with less dense fibrosis tissue associated with reduced liver injury enzymes and metabolic markers in both the acute and the chronic CCl_4 mice model after testosterone treatment ($P<0.05$). Moreover, testosterone treatments displayed significant decrease in serum IL-6 of 2.4-fold ($p=0.0001$) and 2.3-fold ($p=0.0003$) in the acute and chronic models, respectively ($p=0.002$) and data were

associated with increase in INF-γ release from NK associated with a reduction in their IL-6 receptor expressions ($P<0.05$).

Conclusion: Testosterone has an anti-inflammatory and anti-fibrotic action by storing histology for lowering αSMA and Col III levels as well as decreasing ALT and AST levels. In CCl4-induced mice, testosterone improved the metabolic profile by lowering cholesterol, triglyceride, FBS, and C-peptide levels. Testosterone has an anti-inflammatory impact via decreasing inflammatory cytokine levels (lower IL-6).Testosterone treatment of CCl4-mice restored trNK cell function by increasing NK INF-g and decreasing IL-6 receptor levels. These findings emphasize testosterone's immune-modulatory effects, which are linked to its anti-inflammatory and anti-fibrotic capabilities**.** Our results suggest an anti-inflammatory and anti-fibrotic treatment approach of testosterone for delaying disease progressions.

Keywords: Liver fibrosis; Mice Model; Testosterone; Immune And Metabolic Impact.

Chapter One
Introduction and Theoretical Background

1.1 General Background

Liver diseases around the world, is firmly connected with insulin opposition and metabolic disorder, which is one of the most well-known destinations of malignant growth metastasis, liver fibrosis occurs when the healthy tissue of the liver undergoes scarring. Fibrosis represents the primary stage of hepatic scarring, subsequently, if a larger portion of the liver undergoes scarring, it is referred to as liver cirrhosis, the abnormal buildup of parenchymal cells in the liver that are replaced by fibrous connective tissue is known as hepatic fibrosis.[1] Liver damage can arise from various causes, such as the excessive consumption of alcohol, drug utilisation, exposure to chemical toxins, viral infections, and impairment of the immune system.[2] The treatments for liver fibrosis now focus on either controlling the underlying condition or curing it by steadily reducing inflammation, minimizing oxidative stress, and accelerating collagen decomposition.[3] The liver exhibits sexual dimorphism and carries out various analogous functions in both male and female individuals. Males exhibit a pronounced liver-to-weight ratio and possess enhanced capabilities in alcohol clearance and lipid metabolism. Conversely, females demonstrate an improved capacity for cholesterol metabolism.[4][5]

Carbon tetrachloride is a transparent and unstable liquid that is not flammable and is generated through the reaction of chlorine and chloroform in the presence of light, Cellular damage can arise from two primary mechanisms: The process of covalently attaching reactive intermediates to cellular constituents, or the increased peroxidation of lipids caused by the interaction between free radical intermediates and oxygen, leads to the oxidation of unsaturated fatty acids. This phenomenon leads to the degradation of lipids, particularly unsaturated phospholipids, resulting in internal membrane and plasma membrane damage, The production of free radical CCl_3 and other metabolites generated by cytochrome P450 is responsible for the manifestation of CCl_4 toxicity. Ultimately, they induce cellular damage by modifying the structure of cells through processes such as lipid peroxidation and other mechanisms. The presence of free radicals can lead to the development of serious conditions characterised by the dysfunction of multiple organs.

At the molecular level, the activation of various factors, such as tumour necrosis factor (TNF), transforming growth factor (TGF), -β, and nitric oxide (NO), occurs in the cell upon exposure to CCl4. These factors seem to contribute to the cellular process of apoptosis or fibrosis. Tumour necrosis factor (TNF) induces programmed cell death, known as apoptosis while transforming growth factors (TGFs) direct cells towards the pathological process of fibrosis.[6]

The insult generated by carbon tetrachloride (CCl4) in animal models of liver fibrosis is a manifestation that encompasses key characteristics of human liver fibrosis, including inflammation and regeneration, and fiber production. This model is often used to research acute liver damage, advanced fibrosis, and fibrosis reversal. Furthermore, the CCl4-induced liver fibrosis model is highly repeatable, making it a good choice for drug screening. A singular administration of carbon tetrachloride (CCl4) induces centrizonal necrosis and steatosis, whereas prolonged exposure leads to the development of liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). The direct impact of CCl4 on hepatocytes is manifested through its influence on the permeability of plasma, lysosomal, and mitochondrial membranes. The hepatocytes' mixed-function oxidase system also generates highly reactive free radical metabolites through cytochrome P450, leading to the occurrence of severe centrilobular necrosis.[7] This model has been extensively utilized to study the pathophysiology of fibrosis.[8]

Testosterone is a male sex hormone called an androgen. Available for use, testosterone is bound principally to sex chemical restricting globulin (SHBG) while the unbound, or free testosterone, is the most bioavailable and dynamic structure, which delivered in a man's testicles. Ladies' bodies additionally produce testosterone, however in more modest sums. Testosterone substitution treatment might expand your gamble for prostate malignant growth.[9] Testosterone is classified as a steroid hormone with immunoregulatory properties, enabling it to interact with a range of immune system cells, encompassing both innate and adaptive cell types. When these hormones bind to the androgen receptor, they induce specific responses in both lymphoid and non-lymphoid tissues.[10]

Metabolic syndrome has emerged as a significant global public health issue, exhibiting a strong association with both obesity and the widespread prevalence of diabetes. The various elements comprising Metabolic Syndrome (MetS) constitute a collection of risk

factors that are linked to the progression of cardiovascular disease, a matter of growing international importance. Metabolic syndrome (MetS) is associated with several risk factors, including obesity, dysregulated blood sugar levels, elevated blood pressure, heightened triglyceride levels, and diminished high-density lipoprotein cholesterol levels.[11] Metabolic dysfunction-associated fatty liver disease is a chronic illness marked by hepatic fat buildup in addition to underlying metabolic imbalance.[12] Insulin resistance links MetS with liver fibrosis. Insulin resistance contributes to MetS and visceral adipose tissue mass. The former increases the release of insulin-inhibiting adipocyte mediators such as leptin and resistin. Adiponectin may reduce systemic insulin resistance and liver fibrosis.[13]

Natural killer cells account for 50% of the total amount of hepatic sinusoids.[14], which lymphocyte subsets able to demonstrating a variety of functions, Initially, these cells were characterized by their prominent granular morphology, absence of conventional T and B cell markers, and capacity to eliminate specific tumor cell lines without discernible antigen-specific recognition. The current characterization of their role in innate host defense against various viral and intracellular bacterial infections is of great significance.[15] After being recruited to secondary lymphoid organs, active natural killer (NK) cells release interferon and stimulate a type I helper T cell response in vivo [16] Additionally, natural killer (NK) cells have the ability to impact the biological activity of mononuclear phagocytes, resulting in heightened phagocytic activity, enhanced bacterial elimination, and increased production of nitric oxide, interleukin IL-6, and IL-12 by macrophages. NK cells were found to enhance the synthesis of IL-6 by Kupffer cells through an IFN-dependent mechanism, both in vivo and in vitro.[15] In a research investigation of people suffering from severe heart failure reported the elevated IL6 levels were associated with decreased NK cell activity.[17] Topical injection of IL6 to patients with cancer suppressed NK cell activation.[18]

(IL-6) plays a pivotal role in triggering the acute phase response and safeguarding against infections in the liver. (IL-6) is a crucial factor in the maintenance of hepatocytes and possesses potent hepatocyte-stimulating properties. [19] The production of this protein is contributed by various cell types, including endothelial cells, monocytes, macrophages, T cells, and fibroblasts. The synthesis and release of interleukin-6 (IL-6) are heightened when cells are stimulated by interleukin-1 (IL-1) or tumour necrosis factor (TNF), or

when the Toll-like receptor-4 is activated by lipopolysaccharide.[20] The main mediator of the acute phase response, known as C-reactive protein (CRP), is primarily synthesised in the liver through IL-6-dependent production. The administration of human recombinant IL-6 has been shown to induce gluconeogenesis, resulting in elevated blood glucose levels, and subsequently triggering compensatory hyperinsulinemia in animal models of glucose metabolism. [21] Humans have shown similar metabolic reactions after receiving subcutaneous recombinant IL-6.[22] Inflammation may have a role in the genesis of diabetes, according to the results of cross-sectional research; multiple studies have shown increased levels of IL-6 and CRP in persons with both insulin resistance syndrome characteristics and clinically overt type 2 DM.[23][24] The IL-6 molecule has been found to impede the hepatic production and distribution of glucose to the peripheral tissues. This effect is achieved through the expression of glucose-6 phosphatase, a key enzyme involved in gluconeogenesis, in a STAT3-dependent manner.[25] CD8 T and NK cells have been shown to have their IFN expression and cytotoxic responses dampened by IL-6.[26] Perforin and granzyme B expression were shown to be reduced in IL-6-treated human NK cells, leading to decreased cytotoxicity. This mechanism increases SOCS3 synthesis and JAK3/STAT3 phosphorylation. IL-6 suppresses NK-cell cytotoxicity by mediating the expression of programmed death-ligand (PD-L) 1 on NK cells, although inhibiting IL-6 boosted NK-cell cytotoxicity.[27] The cytokine Interleukin-6 (IL-6) is essential for the homeostasis of hepatocytes and exhibits strong mitogen activity towards these cells. The process in question is not only linked to the regeneration of the liver but also to its metabolic functioning. Sustained activation of the IL-6 signaling pathway has deleterious effects on hepatic tissue and can ultimately culminate in the formation of hepatocellular carcinoma. In order to initiate intracellular signaling, IL-6 has the capability to bind to the signal-transducing component glycoprotein 130 located on target cells, either in combination with the bound to the membrane or soluble IL-6 receptor.[19]

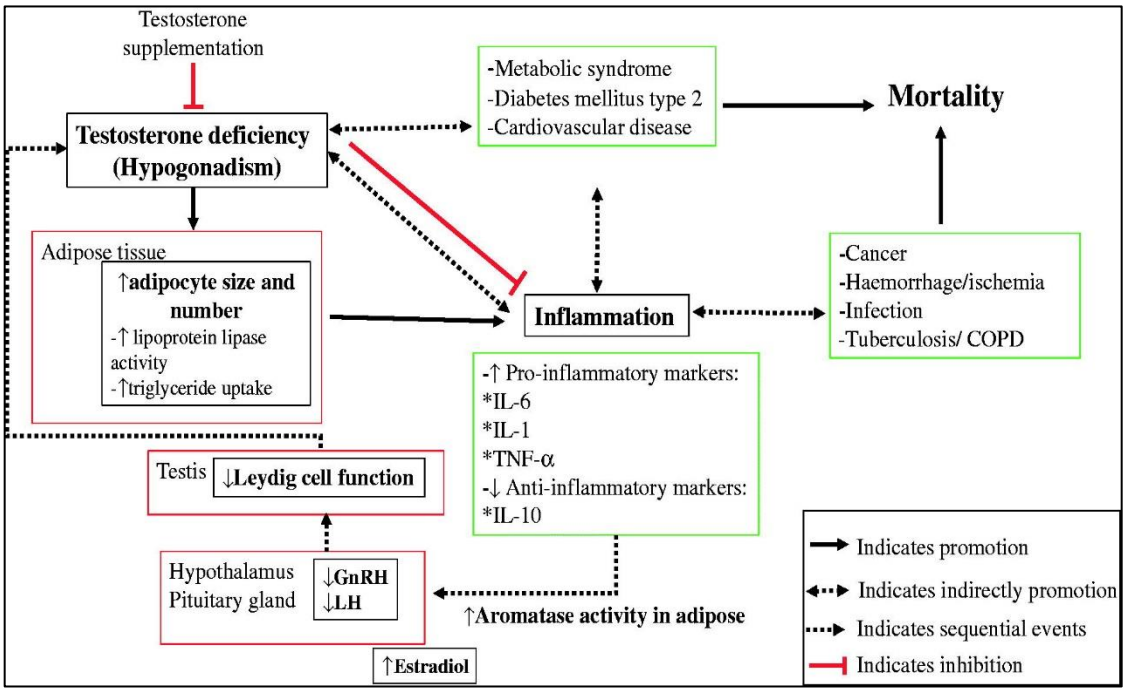
Testosterone's Inflammatory Action Mechanism, testosterone and obesity interact, Furthermore, it has been determined that Testosterone levels correlate inversely with fat percentage. The impact of androgens on body fat is characterized by a consistent increase in lipolysis stimulation and a reduction in adipose tissue lipoprotein lipase activity. This effect is observed in both males and females, as androgens prevent the conversion of

subcutaneous and visceral preadipocytes into adipocytes. Consequently, adipogenesisis significantly restricted by androgens.[28] Demonstrated that elevated levels of testosterone lead to a decrease in adipose tissue accumulation, as well as an enhancement in insulin resistance and glucose tolerance among both male and female individuals.[29], the administration of testosterone therapy has been found to exhibit a notable anti-inflammatory impact, primarily ascribed to the decrease in adipose tissue, which is recognised as a prominent origin of diverse inflammatory cytokines.

The intricate interplay among testosterone, aggression, and illness is significantly influenced by adipose tissue, which enhances the activity of aromatase (an enzyme responsible for the complete conversion of testosterone to estradiol)[22], as depicted in the associated figures.

Figure 1

Interactions between testosterone and inflammation



Note : N.-V. Mohamad *et al.*, "The relationship between circulating testosterone and inflammatory cytokines in men," *Aging Male*, vol. 22, no. 2, pp. 129–140, Apr. 2019

Crisostomo et.al conducted a study wherein they observed that testosterone administration prior to ischemia in castrated male and female rats resulted in an increase in inflammation. The observed phenomenon can be ascribed to the upregulation of active p38 and SPAK/JNK, signalling proteins that are linked to myocardial inflammation. The

findings from in vitro investigations indicate that the introduction of testosterone replacement therapy in macrophages derived from orchiectomies mice significantly decreased the production of inflammatory cytokines in comparison to orchidectomized controls, owing to the reduced expression of toll-like receptor-4. The Toll-like receptors play a crucial role in initiating the immune response to the formation of cancer. The findings of this study indicate that testosterone has the potential to mitigate the inflammatory response in mice, thereby conferring potential benefits to a range of immunological processes. Corcoran et al. found that the expression of the TNF-gene and protein in human monocyte-derived macrophages isolated from both males and females was dramatically inhibited at both physiological (10 nM) and pharmacological (100 nM) doses of testosterone.[30]

This research used a mouse model of testosterone-induced liver fibrosis to investigate the metabolic, immunological, antifibrotic, and antioxidant effects of testosterone. Both short- and long-term versions of liver damage were studied in this experiment.

1.2 Theoretical background

1.2.1 Testosterone

1.2.1.1 Definition

Testosterone, an androgen having C-4-C-5 double bond unsaturation and 17beta-hydroxy and 3-oxo groups, Testosterone is synthesised from cholesterol. The testes are responsible for the majority of testosterone production, although the ovaries' theca cells, the adrenal cortex' zona reticulosa, and the placenta all contribute to the hormone's synthesis as well. In women, testosterone is synthesised from estradiol in the liver, adipose cells, and other peripheral organs via a process called reverse aromatization.[31]

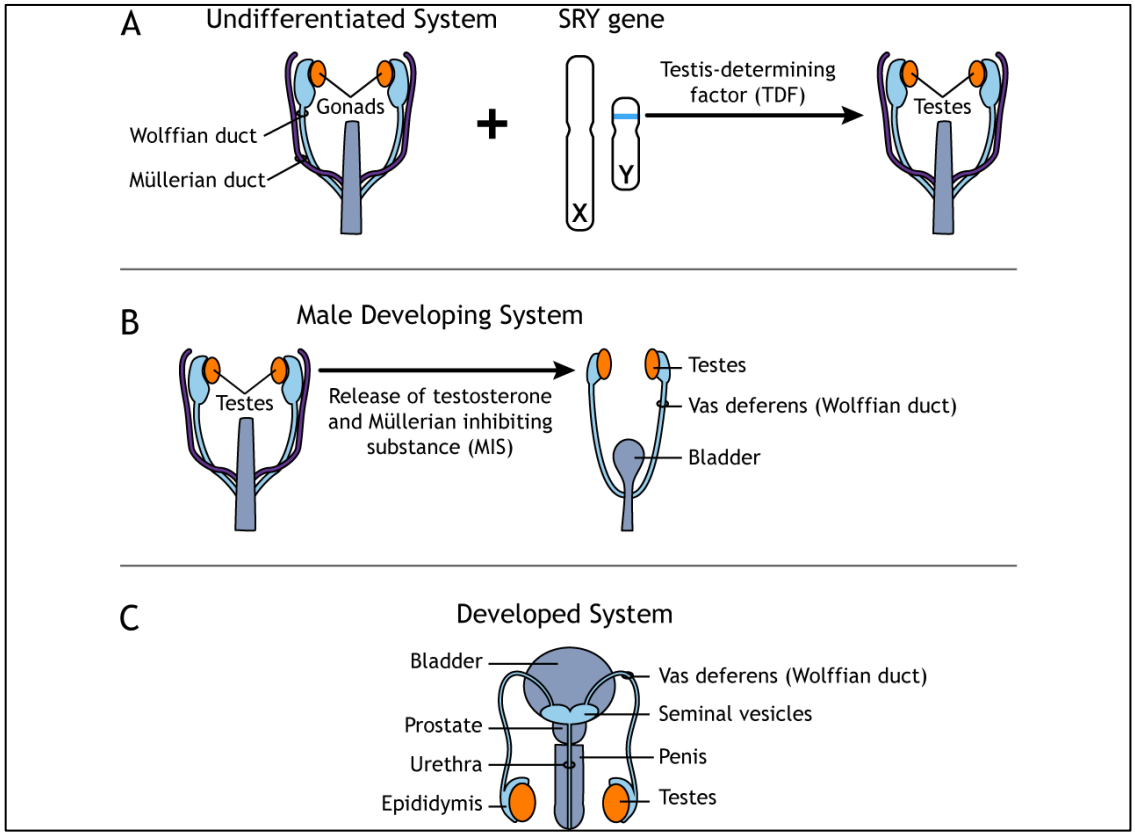
Which is primary male hormone, regulates male sex differentiation, spermatogenesis, fertility, and the process of masculinization involves the development of male sexual characteristics. According to the research, the impact of testosterone is initially observed during the foetal stage. It is a commonly known fact that during the initial six weeks of development, both males and females possess similar reproductive tissues. The onset of testicular development at approximately the seventh week of gestation is attributed to the presence of a sex-determining gene located on the Y chromosome. The seminiferous

tubules are derived from the Sertoli cells that are found within the testis cords during the developmental stage of the foetus. The Fallopian tubes, uterus, and upper segment of the vagina are subject to a process of regression. Sertoli cells are known to produce Mullerian-inhibiting substance (MIS), which is typically associated with female-specific Mullerian structures. The facilitation of male urogenital tract development is attributed to the differentiation of the structures within the Wolffian duct. This process is supported by the migration of Leydig cells and endothelial cells into the gonad during foetal development, leading to the production of testosterone. In the periphery, testosterone undergoes conversion to dihydrotestosterone (DHT), a hormone that facilitates the growth and maturation of the male external genitalia and prostate. The translocation of the testes via the inguinal canal, occurring during the last two months of the foetal stage, is also facilitated by testosterone. The formation of ovaries occurs in an embryonic organism that is devoid of a Y chromosome and, as a result, lacks the SRY gene. The Wolffian ducts do not form because the fetal ovaries do not make enough testosterone. Additionally, these individuals lack the presence of Mullerian inhibiting substance (MIS), which consequently promotes the development and expansion of the Mullerian ducts as well as other anatomical structures associated with female reproductive functions.[32]

The process through which a person grows into a male or female is known as sexual differentiation to present the basic notions of reproductive development.[33]

Figure 2

The undifferentiated gonadal system exhibits identical characteristics in individuals of both sexes



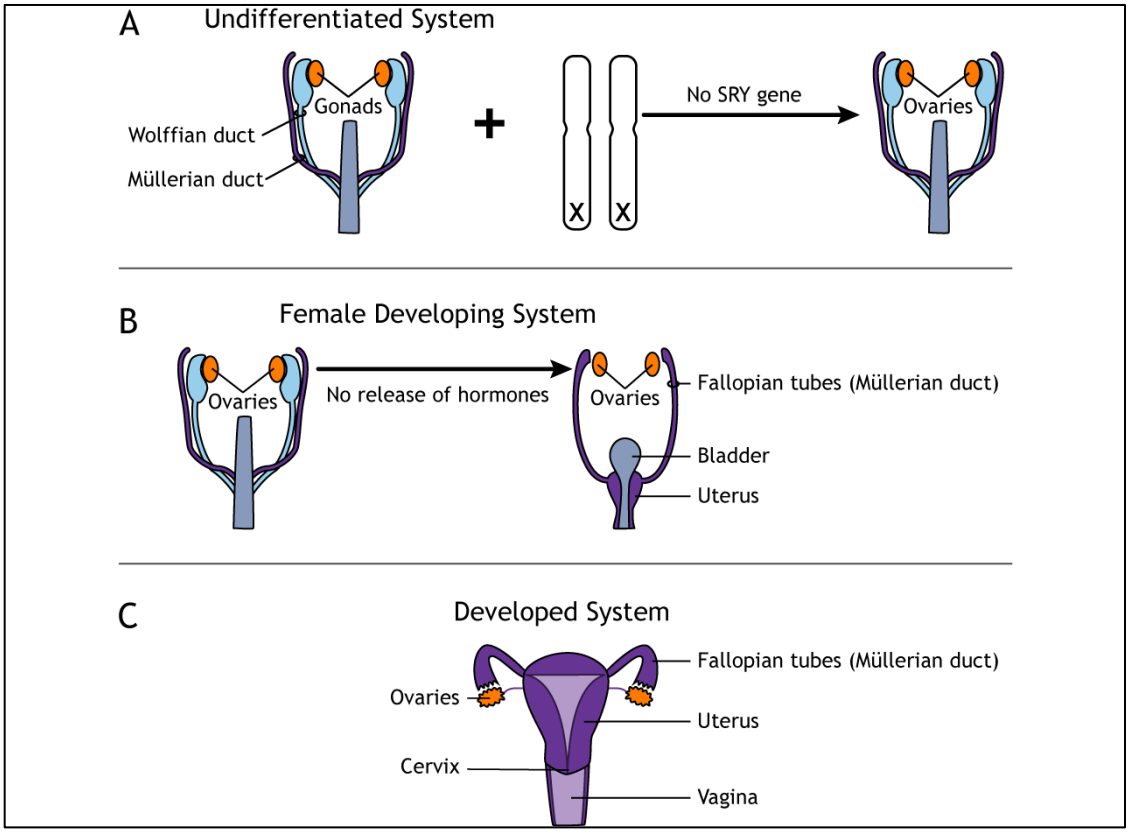
Note: M. Jacobson, "Foundations of Neuroscience," *Found. Neurosci.*, 1993.

The SRY gene, located on the Y chromosome, exhibits continuous activity during male development, leading to the production of testis-determining factors and subsequent differentiation of the gonads into testes. B. During the process, the testes commence the secretion of testosterone and Müllerian inhibitory substance, resulting in the regression of the Wolffian ducts into the vas deferens, seminal vesicles, and epididymis, while the Müllerian ducts undergo degeneration. C. The presence of testosterone is associated with the promotion of penile and prostate gland growth in the fully developed male reproductive system.

In cases where females lack the presence of the SRY gene and associated hormones, the gonads undergo differentiation into ovaries. Additionally, the Müllerian ducts undergo development, giving rise to the fallopian tubes, uterus, and vagina, while the Wolffian ducts experience degeneration.

Figure 3

The undifferentiated gonadal system exhibits identical characteristics in individuals of both sexes



Note: M. Jacobson, "Foundations of Neuroscience," *Found. Neurosci.*, 1993.

During the period of gestation between the sixth and twelfth weeks, the ovaries develop from the gonadal tissue in the absence of the SRY gene. The lack of ovarian hormone secretion during embryonic development leads to the development of the Müllerian ducts into the fallopian tubes, uterus, and vagina, while the Wolffian ducts undergo regression. The cervix is the lowermost segment of the uterus that separates the uterine cavity from the vaginal canal in a well-developed anatomical structure.

1.2.1.2 Function

Testosterone mediates the fundamental process of sexual development, encompassing spermatogenesis, growth of the testes and penis, and heightened libido. Over the seventh month of gestation, the testes begin to secrete a significant quantity of testosterone, which frequently triggers the descent of the testes towards the scrotum. In male newborns who otherwise seem healthy, testosterone treatment has been demonstrated to improve the rate

of testes descent via the inguinal canals, yet undescended testes that have failed to descend by the age of 4 to 6 months.[35]

Moreover, testosterone regulates the development of secondary male characteristics, which are giving men their masculinity. The physiological changes that occur during male puberty include alterations in hair distribution, vocal characteristics and voice pitch. Testosterone has been observed to promote tissue growth at the epiphyseal plate during the early stages of development, resulting in growth spurts and anabolic effects, ultimately leading to plate closure during later stages of puberty).The process of skeletal muscle growth is regarded as a secondary sexual characteristic, which is stimulated by (testosterone that enhances protein synthesis). In addition, it has been observed that the hormone testosterone possesses the ability to induce erythropoiesis, leading to an elevated hematocrit level in males in comparison to females. The decline in testosterone levels among ageing men is often associated with a reduction in testicular size, a decrease in sexual drive, a loss of muscle mass, an increase in body fat, and impaired erythropoietin function, which may contribute to the onset of anaemia.[35]

1.2.1.3 Mechanism

The hypothalamic-pituitary-gonadal axis is a crucial component in the regulation of gonadal function and testosterone levels during the pubertal phase. The secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary gland is induced by the hormones of the hypothalamic and hypothalamic hypophyseal portal system. There are two gonadotropin hormones, namely luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which possess the capability to exert their effects on gonadal receptors located in the systemic circulation.[36]

During the entire reproductive lifespan of a male, the hypothalamus pulse-releases GnRH in a pulsatile manner at intervals of one to three hours. Despite their intermittent release, the mean plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) remain relatively constant from puberty, when concentrations rise, to 20–29 years, when they peak and then gradually decline. Pre-pubertal deficiency in testosterone levels is indicative of reduced gonadotropin and GnRH synthesis.[36]

By converting cholesterol, testicular Leydig cells help make testosterone. LH controls the first phase. Dehydroepiandrosterone (DHEA) and androstenedione are pivotal intermediates within this mechanism. The enzymatic function of 17-beta-hydroxysteroid dehydrogenase enables the transformation of androstenedione into testosterone. The majority of testosterone is primarily bound to two plasma proteins, namely albumin and sex hormone-binding globulin. The human body acquires an excessive amount of testosterone hormone primarily from the source of protein-bound testosterone. The tissues most notably affected by the low levels of free testosterone in the circulatory system encompass the prostate gland, seminal vesicles, bone, and muscle. The enzymatic conversion of testosterone to dihydrotestosterone is mediated by the biological catalyst called 5-alpha-reductase. Testosterone and dihydrotestosterone have the ability to bind to cellular receptors in order to modulate the expression of proteins.[37]

1.2.1.4 Testosterone's Anti-Inflammatory Properties

The presence of metabolic syndrome, cardiovascular disease, and a heightened risk of mortality was found to be associated with decreased levels of plasma testosterone (T). The regulation of adipogenesis and metabolic processes of glucose and lipids are significantly influenced by the variable testosterone (T). Adipocytes are recognized as the principal source of prominent adipokines that trigger inflammation and chronic pathologies. Testosterone affects adipose tissue development and adipocytokines such as leptin, TNF, IL-6, and IL-1. Testosterone suppresses them, while also exhibiting a positive correlation with adiponectin levels. Conversely, a low level of testosterone is linked to heightened expression of inflammatory markers. Further investigation is warranted to examine the role of testosterone (T) in the mechanisms underlying the production and regulation of proinflammatory cytokines in conjunction with weight loss and exercise interventions.[38]

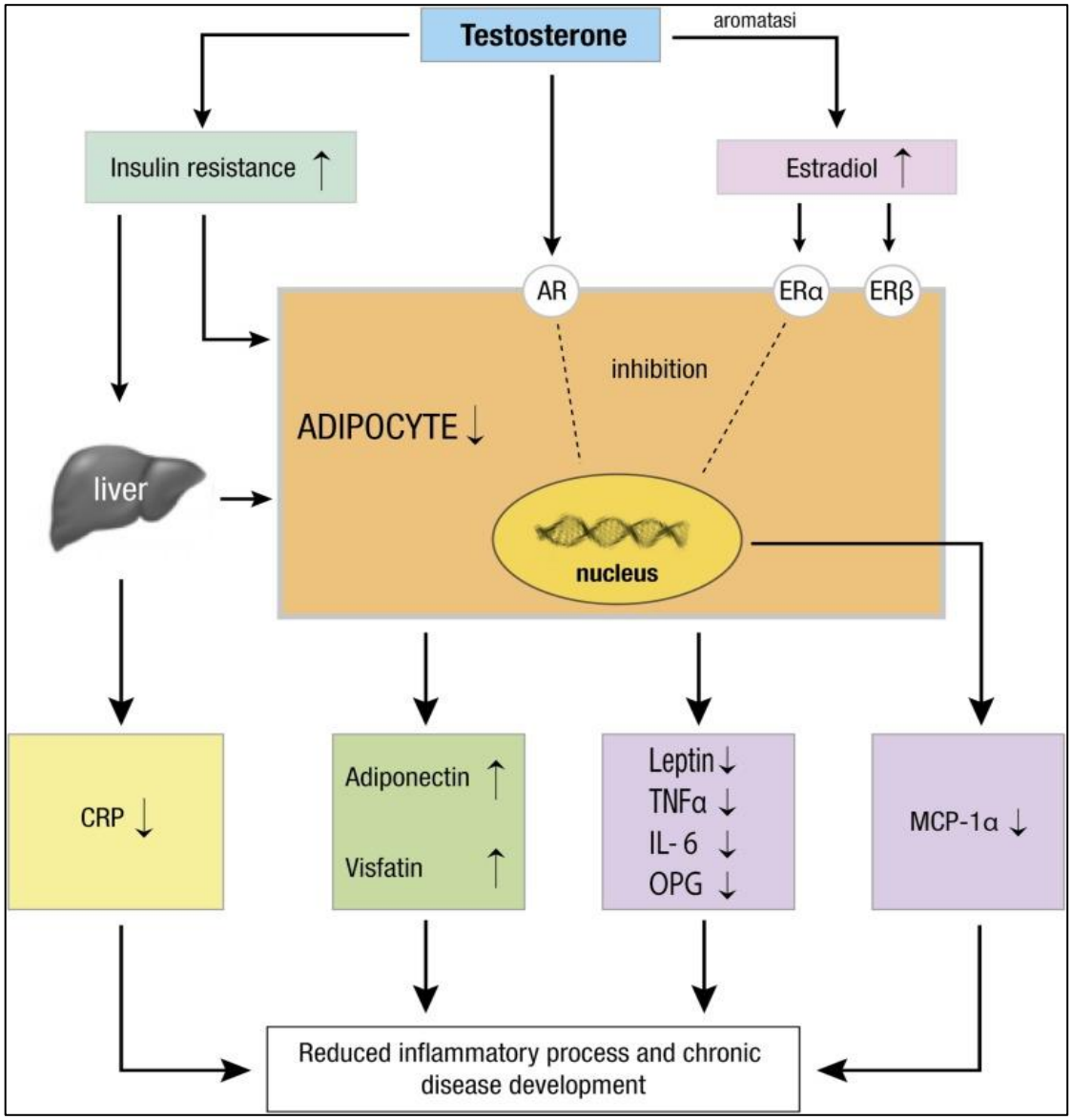
The pathogenesis of cardiovascular disease (CVD) is significantly influenced by heightened levels of proinflammatory cytokines, whereas testosterone (T) therapy has demonstrated the ability to alleviate the pathophysiological markers and clinical manifestations of CVD. Individuals with diminished testosterone levels and individuals who are classified as overweight have an increased propensity to manifest symptoms associated with an inflammatory condition triggered by proinflammatory cytokines. Adipokines may play a role in the mediation of insulin resistance. The most important of

the adipokines implicated include adiponectin, leptin, resistin, visfatin, chemerin, tumour necrosis factor-, interleukin-1, IL-1, IL-6, IL-8, IL-10, plasminogen activator inhibitor-1, monocyte chemoattractant protein-1, and retinol-binding protein-4.[38]

The impact of androgen on adipose tissue is manifested through the secretion of diverse adipocytokines, as Figured in Fig. 3.

Figure 4

The effects of androgen on body fat are reflected in the release of several adipocytokines



Note: V. E. Bianchi, "The Anti-Inflammatory Effects of Testosterone.," *J. Endocr. Soc.*,

Testosterone has various anti-inflammatory actions. To begin with, T has the capacity to limit the proliferation of adipose tissue and decrease both the size and metabolic activity of adipocytes. Testosterone (T), following the process of aromatization into estradiol, has the potential to activate androgen receptors (AR) and estrogen receptors (ER). These interventions contribute to the regulation of adipocytes by reducing the secretion of adipokines such as IL-6, TNF- γ, OPG, MCP-1, and leptin, while concurrently enhancing the synthesis of adiponectin and visfatin, both of which exhibit anti-inflammatory properties. Additionally, T has been found to enhance insulin sensitivity while reducing liver C-reactive protein (CRP) levels. Generally, it leads to a decrease in inflammation and the prevalence of chronic illnesses.

1.2.1.5 Natural Ways to Improve Testosterone in a Man

A considerable number of males exhibit low levels of testosterone and are currently anticipating a prescription for testosterone supplementation. Testosterone plays a crucial role in various aspects of male physiology, including sexual function, physical performance, muscular development, bone density, and regulation of mood. Notwithstanding the potential benefits of testosterone therapy, there are negative effects-documented adverse consequences such as infertility, dependence on lifelong supplementation, elevated blood viscosity, and certain studies indicating an augmented likelihood of experiencing heart attack and stroke. The potential hazards can be significantly reduced and potentially eliminated altogether through the natural elevation of testosterone levels. Prompt administration of testosterone may be necessary for certain individuals. Thus, a complete testicular assessment must include a physical examination, blood analysis, and a review of the patient's medical history, including any past testicular injury, ectomy, radiation exposure, or testosterone use. Prior to determining the appropriate course of therapy, it is imperative to conduct a fundamental series of laboratory tests. [39]

A widely accepted notion posits that an enhancement in overall health leads to a commensurate increase in testosterone levels. The hormone testosterone is responsible for promoting anabolism in the body. However, adverse health conditions and physiological stressors can lead to a reduction in their levels. In order to increase levels of testosterone. [39]

- Reduce stress – One frequently disregarded element, yet crucial to acknowledge for its significant impact on the expression of diminished testosterone levels. Be a complex and multifaceted phenomenon that involves various bodily systems and processes elicit a significant impact on the human body, resulting in the secretion of cortisol, a hormone that exhibits antagonistic effects towards testosterone.
- Sleep is another extremely important impact on testosterone. The majority of testosterone generated in a male occurs during deep sleep. As a result, both the quantity and quality of sleep are crucial.
- Decrease body fat - The increasing of body fat and conversion of testosterone to estrogen: thus, it is imperative to uphold a healthy body weight. It is important to avoid fad or crash diets as they have been found to significantly reduce testosterone levels. Therefore, it is not advisable to engage in starvation practices. In conditions of relative starvation, the human body experiences a significant reduction in the production of testosterone. Under circumstances of moderate food scarcity, the human body experiences a notable decrease in the synthesis of testosterone.
- Diet - Another important factor in testosterone production is diet. Diet guidelines might fill a whole book; therefore, this is simply a basic introduction. Certain meals, such as salmon and eggs, provide the essential elements required by the body to produce testosterone. Foods high in zinc and magnesium are further examples. Furthermore, some vegetables, such as broccoli, cauliflower, and cabbage, are required to eliminate estrogens from the body.
- Exercise - Exercise has been shown to increase testosterone levels.
- Augment the consumption of vitamin D.

Vitamin D is a vital micronutrient that is known to exert a significant influence on various facets of human health. Despite its significant importance, a deficiency in this nutrient is observed in up to one billion individuals globally. According to some studies, low vitamin D levels may be associated with low testosterone levels. [40]

1.2.1.6 Testosterone and immunity

Testosterone has been found to exert a wide range of effects on the immune system in males. Research findings indicate that heightened concentrations of testosterone have the potential to impede the functioning of the immune system in specific situations. In alternative manners, testosterone has the potential to augment or optimise the immune response, whereas other research indicates that testosterone has minimal or negligible impact on immunological well-being in males who are otherwise in good health.

Three potential mediating pathways for testosterone-mediated immunoredistribution have been proposed. To begin, testosterone may directly stimulate immunoredistribution by binding to receptors on leukocytes or endothelial cells, causing migration to specified organs, Second, testosterone may increase corticosteroid levels, causing immunoredistributiol, Ketterson et al. [41] and Ketterson and Nolan.[42]reported that experimentally increased testosterone generates higher corticosterone levels in dark-eyed juncos, Finally, high testosterone levels may simply correspond with high corticosteroid levels as a result of the stress of male-male rivalry or wooing.[43]

In mature males, DHT only makes up 10% of the concentration of testosterone, the androgen with the highest concentration. Thus, full testicular assessment by physical examination, and blood analysis, Only 2% of testosterone in males is free, 30% has a high-affinity binding site for the sex hormone binding globulin (SHBG), and the rest has a lesser affinity for albumin and other proteins. and a complete medical history, including testicular injury, ectomy, radiation exposure, and testosterone use, is essential. The biological functionality of androgens relies on the existence of unbound testosterone, a process that is governed by androgen-binding proteins.[45]

The concentration of androgens is a determining factor in the prevalence of androgen receptors (ARs), which is observed to be higher in males than in females.[6] The abundance of androgen receptors (AR) in macrophages, T lymphocytes, and B lymphocytes highlights the significance of comprehending the control of the immune response through the diversity of androgens and their affinities for AR.[47]

1.2.2 Liver Fibrosis

1.2.2.1 Definition

The dominant manifestation of chronic liver disease is marked by hepatic fibrosis, which is characterised by an aberrant buildup of extracellular matrix proteins, specifically collagen. Liver transplantation is frequently indicated for the management of the various complications arising from advanced liver fibrosis, encompassing conditions such as cirrhosis, liver failure, and portal hypertension. The cellular and molecular underpinnings of liver fibrosis have been better understood in recent years. Injured liver collagen is produced by activated hepatic stellate cells, portal fibroblasts, and bone marrow-derived myofibroblasts. Fibogenic cytokines including TGF-1, angiotensin II, and leptin stimulate these cells.[48]

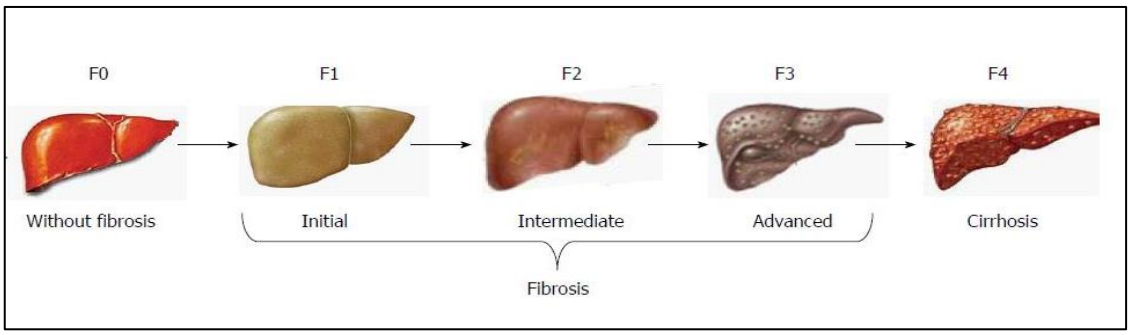
The fibrosis stages classified according MITAVIR score from F0 to F4:

- F0: no fibrosis
- F1: portal fibrosis without septa
- F2: portal fibrosis with few septa
- F3: numerous septa without cirrhosis
- F4: cirrhosis

A lower number may suggest less harm, whereas a larger score may imply more.[49]

Figure 5

The liver fibrosis stages classified according MITAVIR score from F0 to F4



Note: U. R. Acharya *et al.*, Automated detection and classification of liver fibrosis stages using contourlet transform and nonlinear features.

Acute liver failure is characterised by the abrupt onset of liver dysfunction, typically occurring within a short timeframe of days or weeks, in individuals without a history of liver disease. The primary etiological factors include the hepatitis virus and certain medications, such as paracetamol. The incidence of acute liver failure is lower compared to that of chronic liver failure, which manifests gradually over a period of time. This condition is alternatively referred to as fulminant hepatic failure. In many cases, medication can cure the condition; nevertheless, a liver transplant may be the only choice.[50]

Chronic liver fibrosis is distinguished by an ongoing sequence of inflammatory, destructive, and regenerative events within the liver parenchyma, leading to the disturbance of liver structure, the formation of numerous nodules, the reorganization of blood vessels, the development of new blood vessels, and the deposition of extracellular matrix. The development of fibrosis is attributed to the activation and accumulation of stellate cells and fibroblasts, whereas the restoration of parenchymal tissue is reliant on the presence and activity of hepatic stem cells.[51]

1.2.2.2 Liver Immunity and Fibrosis Resolution

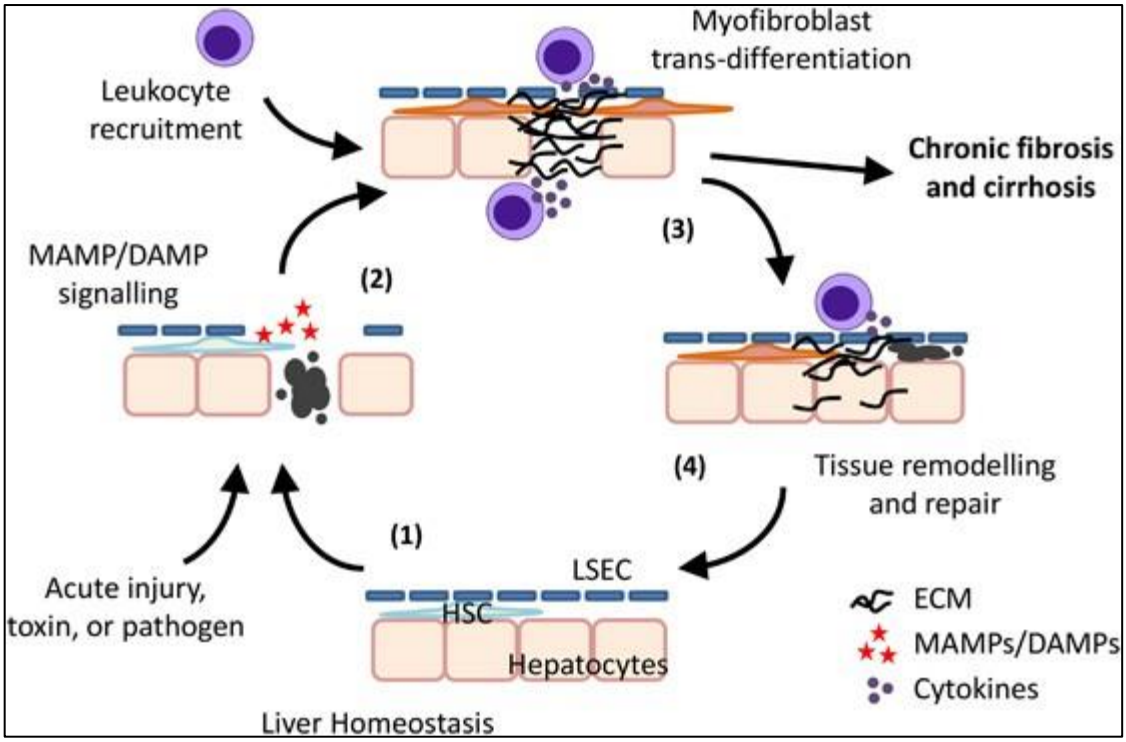
The immune system in the liver has to be able to respond rapidly and precisely to tumor cells and dangerous microorganisms while keeping the environment generally tolerogenic. The liver is a common target for many infectious agents such as viruses, bacteria, and parasites.[52] The ability of local immunity to detect and eradicate these hepatotropic diseases is crucial. During acute liver injury, activated KCs may produce high levels of chemokines like MIP-1 and RANTES and pro-inflammatory cytokines including IL-1, IL-6, and TNF-- γ.[53] In healthy adult livers, CD141+ myeloid DCs with significant pro-inflammatory characteristics may drive T cells to produce IFN-γ and IL-17.[54] In situations where conventional CD4+ T cell support is lacking, T cells that have been primed in the liver have the capacity to proliferate and differentiate into functional T effector cells. These cells are capable of facilitating the clearance of pathogens in an inflammatory environment.

Acute liver inflammation recruits and activates leukocytes and starts fibrotic responses, which are essential processes.[55] Resolving acute fibrosis protects surviving hepatocytes by decreasing pro-apoptotic signalling and boosting toxicity tolerance. Leukocytes'

production of inflammatory cytokines and growth factors following damage migration governs fibrosis. Examples of cytokines include TNF, interleukin-6, platelet-derived growth factor and TGF. Cytokines play a crucial role in the activation and proliferation of hematopoietic stem cells (HSCs). These entities are well-known for their ability to produce various components of the extracellular matrix, which is the first kind of collagen and smooth muscle actin. [56] Pathological liver fibrosis is well known. However, liver trauma healing and tissue homeostasis need fibrosis resolution. The clinical significance of fibrosis arises solely from its ability to alter tissue structure and function due to inflammation that is either dysregulated or uncontrolled.[57]

Figure 6

In reaction to liver injury, the liver maintains local homeostasis



Note: M. W. Robinson, C. Harmon, and C. O'Farrelly, Liver immunology and its role in inflammation and homeostasis.

The activation of inflammatory mechanisms is of utmost importance in maintaining liver homeostasis in the context of cell death or infection. Hepatocytes, when subjected to acute injury, cell death, or infection, create DAMPs and PAMPs. (1) Hepatocytes, HSCs, and liver immune cells detect and stimulate the chemicals under investigation. After being activated, cells release inflammatory mediators that aid in the recruitment of leukocytes

and induce the trans-differentiation of hematopoietic stem cells (HSCs) into myofibroblasts. The myofibroblasts then initiate the process of fibrosis by synthesising constituents of the extracellular matrix. (2) During the inflammatory process, leukocytes are recruited, leading to the synthesis of pro-resolving factors and the induction of myofibroblast apoptosis. (3) This facilitates tissue regeneration and the restoration of homeostasis. (4) In the event of the lack of a resolution phase, extended inflammation results in the gradual progression of hepatic fibrosis.

In acute liver fibrosis, macrophages release pro-resolution chemicals that upregulate matrix metalloproteinase, while concurrently inhibiting the activity of matrix metalloproteinase inhibitors. [58] Simultaneously, the cytotoxic effects exerted by natural killer cells against hepatic stellate cells play a role in the modulation of fibrosis in the liver. The process of transdifferentiation of hepatic stellate cells (HSCs) into myofibroblasts is associated with alterations in the equilibrium of activating and inhibitory ligands for cell receptors. This alteration facilitates the eradication of the aforementioned entities by natural killer (NK) cells via pathways that are dependent on TRAIL, FasL, and NKG2D.[59] The intricate interplay among various immune cell populations that regulate the mechanism of tissue repair has led to the conceptualization that wound healing is an integral element of the innate immune response to tissue damage, as postulated by numerous scholars.

Kupffer cells (KCs) are recognized for playing a pivotal role in the process of liver regeneration, primarily through the secretion of cytokines like interleukin-6 (IL-6) and tumour necrosis factor (TNF). These cytokines actively stimulate the proliferation of hepatocytes. On the other hand, previous studies have demonstrated that the reduction of Kupffer cells (KCs) can hinder the process of liver regeneration that follows. Neutrophil migration to the liver, triggered by inflammatory signals of the activation of KCs is facilitated in an ICAM-1-dependent manner. The activation of Kupffer cells and the stimulation of hepatocyte proliferation following partial hepatectomy are significantly dependent on the complement proteins C3 and C5. Furthermore, there exist favorable characteristics that facilitate the process of regeneration, it is noteworthy that certain immune cell populations within the liver may hinder this process. Natural killer cell elimination reduces interferon production, which reduces hepatocyte cell cycle arrest and proliferation, allowing liver regeneration. In mice that contain the hepatitis B virus

transgene, natural killer T (NKT) cell depletion increases liver regeneration via decreasing IFN and TNF production. The hepatic immune cell populations function as a negative feedback loop, modulating the hepatic regeneration process and restoring homeostasis.[57]

Numerous hepatic disorders are distinguished by either persistent or heightened inflammation. Chronic infection, tissue injury, excessive alcohol or fat consumption, or cancer growth is known to activate innate immune detection mechanisms in a chronic manner, resulting in the hallmark hallmarks of pathological liver inflammation. HSC-derived myofibroblasts remain active in response to inflammatory signals from both immune and non-hematopoietic cell types. [60] This reduces the effectiveness of both myofibroblast senescence and NK cell-induced myofibroblast death. Loss of hepatic tolerogenic systems is caused by the excessive inflammation already present in a diseased liver. The liver is attractive to inflammatory monocyte-derived macrophages, Factors that induce fibrosis can impede the capacity of Kupffer cells to facilitate the maturation of T regulatory cells.[57]

1.2.2.2.1 Innate immune cells in liver fibrosis

- Kupffer cells (KCs) make up 15% of liver cells and are self-renewing embryo-derived local macrophages. KCs emit TGF-1, TNF-, MCP-1, and other cytokines that stimulate HSCs during hepatic fibrogenesis. KC depletion by gadolinium chloride reduced CCl4-induced liver fibrosis and TGF expression in rats.[61] KCs are positioned in the sinusoids' core, allowing for close contact with other non-parenchymal hepatic cell types. KCs, in particular, can stimulate HSCs via paracrine processes involving TGF- and PDGF production.[62]
- Neutrophils. In general, hepatic inflammation precedes liver fibrosis; hence, infiltrating leukocytes, particularly neutrophils, can accelerate liver fibrosis progression.[63] In the context of a co-culture system, it was observed that reactive oxygen species derived from neutrophils had a stimulating effect on collagen production in human HSCs.[63] MIP-2 and cytokine-induced neutrophil chemoattractant released by activated rat HSCs may attract neutrophils to the injured liver.[62]

- Dendritic cells (DCs) are antigen-presenting cells that play an important role in modulating both innate and adaptive immune responses.[64] According to recent research, liver DCs have a dual function in liver fibrosis. To begin, unlike other organs, liver DCs have a tolerogenic phenotype and are thus thought to constitute the foundation of immunological tolerance to diverse antigens in the liver. Liver fibrosis changes DC function from tolerogenic to immunogenic, boosting NK and CD8+ cell activity and lowering regulator T cell numbers through TNF production.[65]
- The NK cells. Using mouse models, we and other groups have identified NK cells as essential negative regulatory cells in hepatic fibrogenesis[59]. Activated hepatic stellate cells (HSCs) may be killed by NK cells during liver fibrosis through the action of TNF-related apoptosis ligand (TRAIL) and interferon via the retinoic acid early inducible gen-1/NKG2D or activating/inhibitor killer immunoglobulin receptor/MHC class I-dependent pathways.[59] Furthermore, previous study has shown that human NK cells may destroy human HSCs in TRAIL-, Fas-ligand-, and NKG2D-dependent ways, preventing liver fibrosis in patients.[66] In the context of advanced liver fibrosis, it has been observed that intermediately activated hepatic stellate cells (HSCs) possess the ability to evade natural killer (NK) cell-induced death and apoptosis induced by interferon (IFN). This evasion is facilitated by the production of transforming growth factor (TGF) through retinoic acid-mediated pathways, as well as the upregulation of suppressor of cytokine signalling 1 expression. This phenomenon is not observed in early activated HSCs.[67]
- Cytokines, including IL-6, IL-22, IL-33, TGF-, and TNF, are a class of regulatory peptides that are synthesized by various nucleated cell types in the human body, including but not limited to monocytes/macrophages, fibroblasts, epithelial cells, and lymphocytes. Newly published study has unveiled a peculiar association between the process of liver regeneration and injury. Certain cytokines have been observed to facilitate the regeneration of liver tissue in response to damage, while others have been found to induce apoptosis and necrosis of liver cells, as well as fibrosis, cholestasis, and inflammation of the liver. The occurrence of this phenomenon has been observed in hepatic tissues, wherein ischemic preconditioning has been observed to provide protection against subsequent ischemia-induced damage.

Additionally, instances of liver injury resulting from ischemia-reperfusion-induced apoptosis have also been documented.[68]

1.2.2.2.2 Adaptive immune cells in liver fibrosis

A growing body of evidence suggests that the adaptive immune system plays a substantial role in the pathogenesis and advancement of liver fibrosis. T helper 2 cells produce profibrotic cytokines such as IL-4. In contrast, Th1 cells are known to secrete cytokines with antifibrotic properties, including interferon (IFN). The Th1/Th2 ratio affects liver fibrosis.[69] There is a documented association between the occurrence of liver fibrosis and the presence of elevated levels of CD8+T cells, as well as a reduced CD4+/CD8+ ratio specifically within Th1 lymphocytes, in both murine and human subjects. [70]. The observation that collagen deposition was diminished in animals with B cell deficiency in CCl4-induced fibrosis suggests the involvement of B cells in the development of hepatic fibrogenesis.[71] the following are some of the potential methods through which B-cells influence hepatic fibrogenesis. For starters, it's possible that B cells release profibrotic cytokines like IL-6, which contribute to liver fibrosis by activating HSCs and increasing collagen and TIMP production. Second, with B cell depletion, changes in T cell activity may contribute to the development of liver fibrosis. As a result, we must not dismiss B cells as 'bystanders' in the pathophysiology of liver fibrosis.

Ultimately, hepatic fibrosis is the common outcome of all forms of liver damage, irrespective of their origin. Upon liver damage, hepatic stellate cells (HSCs) undergo activation and transdifferentiation into cells resembling myofibroblasts. This process leads to the release of a substantial quantity of extracellular matrix (ECM) proteins and immunoregulatory cytokines. Moreover, a growing body of evidence indicates that various types of immune cells play a significant role in the pathophysiology of liver fibrosis and engage in interactions with hepatic stellate cells (HSCs) to either facilitate or impede the progression of hepatic fibrosis. Despite intensive study and advances in our understanding of liver fibrosis progression, effective anti-fibrotic drugs remain elusive. Further investigation is warranted to elucidate the principal cellular effectors responsible for liver fibrosis and the crucial cytokines that regulate the fibrotic process. This will facilitate the identification of innovative therapeutic targets and the development of cell-based immunotherapies for the management of liver fibrosis.

Natural history and diagnosis of liver fibrosis

The majority of morbidity and mortality is typically observed subsequent to the onset of cirrhosis, and the manifestation of liver fibrosis generally occurs gradually and inconspicuously. After an interval of 15 to 20 years, cirrhosis progresses in most individuals. Ascites, renal failure, hepatic encephalopathy, and variceal hemorrhage are the main clinical side effects of cirrhosis. Patients with cirrhosis can go years without experiencing any severe effects (compensated cirrhosis). Decompensated cirrhosis has a low survival rate, and liver transplantation is frequently the only recommended treatment.[48]

Cirrhosis may lead to hepatocellular cancer. In HCV-reinfected patients with recurrent severe acute alcoholic hepatitis, subfulminant hepatitis, and fibrosing cholestasis after liver transplantation, liver fibrosis generally progresses quickly to cirrhosis.[72]

A liver biopsy is widely considered the definitive method for assessing liver fibrosis. Histologic testing enables the assessment of the necroinflammatory response degree, fibrosis stage, and the underlying aetiology of liver disease. The fibrosis stage is determined by employing measures such as the Metavir scoring system, which consists of stages I to IV, and the Ishak scoring system, which ranges from stages I to V. Computer-guided morphometric analysis can be used to measure the degree of fibrosis utilizing specific staining of ECM proteins (for instance, Sirius red). The liver biopsy is a procedure that is characterized as invasive and has been reported to elicit pain in 40% of patients, with 0.5% experiencing significant complications.[72]

Numerous suggestions have been put forth concerning the integration of regular laboratory measurements, including platelet count, serum aminotransferase levels, prothrombin time, and concentrations of acute phase proteins. Various proteins, such as the N-terminal propeptide of type III collagen, hyaluronic acid, tissue inhibitor of metalloproteinase type 1 (TIMP-1), and YKL-40, have been recognized as direct indicators of the hepatic fibrogenic process and are employed as surrogate markers for liver fibrosis. Furthermore, the indicators associated with fibrosis may indicate the presence of fibrogenesis in other organs of the body, such as pancreatic fibrosis in individuals with a past of alcoholism. Hepatic fibrosis can be estimated through the utilization of imaging techniques. Modest to severe fibrosis can cause alterations in

hepatic parenchyma, which can be diagnosed by MRI, computed tomography, and ultrasonography. Portal hypertension symptoms indicate liver cirrhosis, as well as by assessing alterations in the liver's echogenicity and nodularity. The reliability of differentiating hepatic steatosis from fibrosis based on increased liver echogenicity is limited due to the high dependence on the operator in ultrasound imaging.[72]

1.2.2.3 Pathogenesis of liver fibrosis

Hepatic fibrosis occurs as a result of the liver's wound-healing response to repeated injury. Parenchymal cells exhibit regenerative properties and undergo replacement of necrotic or apoptotic cells subsequent to an acute liver injury. During this process, only a small amount of ECM is deposited, additionally, an inflammatory response is evident. In the event of persistent hepatic injury, the process of liver regeneration will ultimately be impeded, leading to the substitution of the liver's hepatocytes with an excess of extracellular matrix (ECM), specifically fibrillar collagen. The distribution of fibrous debris may vary depending on the origin of the liver injury. In cases of chronic viral hepatitis and chronic cholestatic diseases, fibrotic tissue is initially observed surrounding the portal tracts. However, in instances of alcohol-induced liver disease, fibrotic tissue is predominantly located in the pericentral and perisinusoidal regions. The development of fibrotic liver diseases, characterized by the formation of collagen bands, progression to bridging fibrosis, and ultimately leading to the development of cirrhosis, occurs as the condition deteriorates.[73]

The primary reservoir for vitamin A within the Disse space of the sound liver consists of hepatic stellate cells (HSCs), which are the main producers of extracellular matrix (ECM) in the injured liver. HSCs undergo activation or trans differentiation into cells as myofibroblasts subsequent to enduring prolonged injury, and acquiring characteristics that include contractility, pro-inflammatory properties, and fibrogenicity. During the course of tissue healing, activated HSCs shift and congregate where they secrete large quantities of ECM and regulate its breakdown. At both the transcriptional and posttranscriptional levels, HSCs control collagen production. The enhanced collagen production in activated HSCs is mediated by higher collagen mRNA stability. In these cells, the posttranscriptional regulation of collagen is governed by the collaborative action of the RNA-binding protein CP2 and stem-loop structures located in the 5′ ends of collagen mRNA.[73]

In addition to HSCs, it is plausible that there are alternative hepatic cell types that possess the capacity to initiate fibrosis. Myofibroblasts, derived from small portal arteries, undergo proliferation in the vicinity of biliary pathways during instances of cholestasis-induced liver fibrosis, thereby initiating the mechanism of collagen accumulation. The response of distinct cell groups to apoptotic stimuli and the expression of specific cell markers exhibit variability between hematopoietic stem cells (HSCs) and portal myofibroblasts. When CD34+CD38- hematopoietic stem cells from human livers undergoing tissue remodeling cultivated with various growth agents, HSCs and myofibroblasts of bone marrow origin are generated. The findings of this study indicate that the bone marrow has the potential to contribute fibrogenic cells to the liver following injury.[73]

1.2.2.4 Pathogenesis of fibrosis in different liver diseases

The aetiology underlying liver fibrosis plays a significant role in its development. The impact of alcohol consumption on gut microbiota, intestinal motility, and the growth of Gram-negative microorganisms has been documented in the context of alcohol-induced liver damage. The activation of Kupffer cells' NF-B is induced by oxidants, resulting in an upregulation of TNF synthesis. The activation of hepatic stellate cells (HSCs) is facilitated by the induction of inflammatory and fibrogenic signals, which is promoted by the stimulation of reactive oxygen species (ROS) and acetaldehyde, the primary byproduct of alcohol metabolism. Upon entering hepatocytes, the Hepatitis C Virus (HCV) successfully evades detection by the immune response mediated by the Human Leukocyte Antigen class II (HLA-II), leading to oxidative damage and activation of inflammatory cells. Moreover, a number of proteins derived from the Hepatitis C Virus (HCV) have been recognized for their ability to effectively induce the inflammatory and fibrogenic functions of hepatic stellate cells (HSCs). T cells, also known as T lymphocytes, are a type of white blood cell that play a crucial role in the immune response. They are a key component of the adaptive immune system and are responsible for recognizing.

Kine's cells are recognized as the principal entities responsible for the sustained injury inflicted upon the bile ducts in chronic cholestatic disorders, such as primary biliary cholangitis (PBC). The production of fibrogenic mediators by biliary cells stimulates the secretion of ECM in adjacent portal myofibroblasts. Activation of perisinusoidal HSCs

leads to the development of fibrotic bands. The root cause of NASH-related liver fibrosis is unclear. Frequently co-occurring are elevated body mass index (BMI), type 2 diabetes, and dyslipidemia.[48]

1.3 Problem statement

Previous research indicates that a significant proportion of males diagnosed with cirrhosis experience a reduction in their serum testosterone levels, with up to 90% of such individuals being affected. Moreover, the rate of decline in testosterone levels tends to increase as the liver disease advances. Recent research has revealed a correlation between decreased levels of testosterone in males suffering from cirrhosis and elevated mortality rates. This correlation remains significant even after accounting for established prognostic factors, such as the Model for End-Stage Liver Disease score. The effectiveness of testosterone therapy for male patients with cirrhosis has been assessed in a restricted number of clinical trials on a small scale. However, the question of whether testosterone provides any benefits remains unresolved, and its impact on liver is still not clear.

1.4 Significance of the study

What effects could testosterone exert on patients with liver injury of fibrosis on a CCL4 mice model?

1.5 Aim of the study

The main purpose of the study was to show the effects of testosterone treatment on immune and metabolic outcomes of liver injury and the mechanism related to its function.

1.6 The secondary objectives of the study

Following testosterone treatments in acute and chronic model of liver injury, the following lab Measures are assessed:

1. Liver injury enzyme (ALT, AST)
2. Blood sugar glucose, insulin, and HOMA-score.
3. Lipid profile (cholesterol, triglyceride, LDL, HDL)
4. Tissue-resident NK cells activity.

1.7 Research Hypothesis and question

- Liver diseases worldwide is closely associated with insulin resistance and metabolic syndrome.
- The immune cells exhibited discernible metabolic variances, characterized by an elevated redox ratio, which suggests a transition to a more glycolytic metabolic profile and high alteration in NK cell.
- Increase collagen, alpha smooth muscle actin and desmin in liver fibrosis.
- Increase liver injury enzyme in liver fibrosis.
- Increase in glucose blood sugar in liver fibrosis.
- High lipedema (TG, cholesterol, LDL, HDL) in liver fibrosis.
- Liver fibrosis cause dysfunction and impairment in immune cell
- Is testosterone treatment modulating:
    - Liver injury enzymes in liver fibrosis?
    - Lipid profile (cholesterol, triglyceride, LDL, HDL) in liver fibrosis?
    - Immune cell activity alterations
    - Metabolic syndrome in liver injury

1.8 Literature Review

Choi et al. (2014) Study**:** Nonalcoholic fatty liver disease is a negative risk factor for prostate cancer recurrence:

There exists a significant association between metabolic syndrome, a medical condition characterized by an increased susceptibility to specific types of cancer, and NAFLD. This research examined whether radical prostatectomy patients with NAFLD had biochemical recurrence (BCR). Two Korean hospitals recruited consecutive radical prostatectomy patients with prostate cancer. The training set (nZ147) and validation set (nZ146) were randomly assigned to these patients. NAFLD, BMI, preoperative prostate-specific antigen levels, and histological abnormalities like GSc were examined for the BCR relationship. NAFLD was identified using unenhanced computed CT or ultrasonography. The survival rates for individuals without biochemical recurrence (BCR) were computed using the

Kaplan-Meier method. Approximately 32 patients, constituting 21.8% of the training set, encountered biochemical recurrence (BCR) within a median follow-up period of 51 months, with an interquartile range of 35 to 65. Upon accounting for atypical GSC in the multivariate analysis, it was determined that the presence of non-alcoholic fatty liver disease (NAFLD) functioned as a distinct and unfavourable prognostic indicator for biochemical recurrence (BCR). The predictive value of non-alcoholic fatty liver disease (NAFLD) remained significant for a longer duration in terms of time-to-biochemical recurrence (BCR) when applied to the validation set. In the subgroup analysis of individuals with non-alcoholic fatty liver disease (NAFLD), the NAFLD fibrosis score emerged as a significant independent negative predictor for B-cell receptor (BCR) response. After radical prostatectomy for prostate cancer, NAFLD may protect against biochemical recurrence (BCR). The mechanism of the protective effect in NAFLD patients needs more study.[74]

Sinclair et al. (2015) Study: Testosterone in men with advanced liver disease: Abnormalities and implications:

Serum testosterone levels are found to be lower in up to 90% of men with cirrhosis and continue to fall as the liver disease worsens. Advanced liver illness shares many characteristics with hypogonadal males, such as sarcopenia, osteoporosis, gynecomastia, and reduced libido. However, it is not fully known how much testosterone deprivation contributes to the symptoms of severe liver disease. In recent studies, evidence has emerged indicating a correlation between low testosterone levels in males diagnosed with cirrhosis and a heightened likelihood of mortality. This association persists even when considering established prognostic factors, such as the Model for End-Stage Liver Disease score. Testosterone treatment in cirrhotic men has been studied in a limited number of clinical trials, with no conclusive evidence regarding its beneficial effects. Nevertheless, studies have shown that testosterone therapy can effectively increase haemoglobin levels, reduce insulin resistance, and enhance muscle mass and bone mineral density in males suffering from structural hypothalamic-pituitary-testicular axis disorders and idiopathic hypogonadism. Recent data suggest that the previously perceived risk of hepatocellular carcinoma in relation to testosterone has been exaggerated, despite initial concerns. Randomized controlled trials are necessary in order to evaluate the

efficacy, effectiveness, and safety of testosterone therapy in individuals diagnosed with cirrhosis, considering the existing persuasive body of evidence.[75]

Jaruvongvanich et al. (2017) Study: Testosterone, Sex Hormone-Binding Globulin and Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis:

A correlation has been observed between diabetes, metabolic syndrome, and endogenous sex hormones. Emerging research indicates that these hormones potentially contribute to the pathogenesis of nonalcoholic fatty liver disease (NAFLD). The current investigation undertook a meta-analysis and systematic review of observational studies, elucidating a noteworthy association between sex hormones and non-alcoholic fatty liver disease (NAFLD). A thorough investigation was carried out on the MEDLINE and EMBASE databases, encompassing data from their inception up until April 2016. This study included observational research on NAFLD and serum total testosterone (TT) and sex hormone-binding globulin (SHBG). The research findings suggest that males who have been diagnosed with HBV-ACLF often display lower levels of serum testosterone, which is linked to a more severe illness and a less favorable prognosis. These result suggest that HBV-ACLF may be caused by low testosterone levels.[76]

Huang et al. (2021) Study: Lower testosterone levels predict increasing severity and worse outcomes of hepatitis B virus-related acute-on-chronic liver failure in males:

Acute liver failure brought on by the hepatitis B virus (HBV-ACLF) is a devastating condition whose pathophysiology is still unknown. Examining how testosterone levels are related to clinical outcomes, stage, and severity ratings for HBV-ACLF is the focus of this study. This observational analysis was conducted at a single center. The research involved the enrollment of 160 male subjects who had been diagnosed with HBV-ACLF, 151 individuals with chronic hepatitis B but did not exhibit liver failure, and 106 healthy individuals serving as controls. Chemi-bioluminescent immunoassay was used to measure androgen levels in blood samples taken in the morning. The main composite outcome was the amount of time that passed before either the patient died or received a liver transplant during the 90 days.

The study revealed that individuals with HBV-ACLF exhibited elevated serum levels of androstenedione in comparison to those with CHB and HC. In addition, it is worth noting that the HBV-ACLF group exhibited significantly lower levels of total testosterone, free

testosterone index, Dehydroepiandrosterone sulphate, and cortisol. There was a correlation between increased stage and severity and decreasing levels of TT, sex hormone-binding globulin, and FTI. The overall result and mortality rate after 90 days were both negatively impacted by low TT. The findings of the multivariate researches have established the role of TT as a distinct predictor of the composite outcome.[77]

Al-Qudimat et al. (2021) Study: Testosterone treatment improves liver function and reduces cardiovascular risk: A long-term prospective study:

This study's goal was to document the relationship between testosterone therapy and cardiovascular illness. Males with hypogonadism (CVD) are at an increased risk of developing nonalcoholic fatty liver disease and hepatic steatosis, which are conditions related to cardiovascular disease. A research investigation was conducted to assess the long-term influence of testosterone undecanoate on hepatic steatosis in a cohort of 496 males with hypogonadism. The study involved the categorization of participants into two distinct groups. The first group, referred to as the control group, consisted of 184 patients who did not receive any form of medication. The second group, known as the treatment group, comprised 312 individuals who were administered TU 1000 mg every 12 weeks. These individuals were closely observed and evaluated over a period of 8 years. According to the research, hypogonadal males who receive long-term testosterone therapy had improved liver function. While a reduction in CVD-related mortality may be linked to the improved physiological and functional state of the liver.[78]

Shuning et al. (2021): Testosterone and Estradiol as Novel Prognostic Indicators for HBV-Related Acute-on-Chronic Liver Failure:

The main aim of this study was to enhance understanding regarding the impact of testosterone and estrogen on the occurrence and prognosis of HBV-ACLF. This prospective study encompassed a cohort of 300 individuals diagnosed with chronic hepatitis B (CHB). Among these participants, 108 had pre-existing HBV-ACLF prior to their admission, while an additional 20 individuals developed HBV-ACLF during their hospitalization. This research examined the predictive value of blood testosterone and estradiol levels in individuals with acute-on-chronic liver failure (ACLF) from heterogeneous demographics, illness severities, and cirrhosis statuses. This research examined these biomarkers' prognostic value in a short period. The group of individuals

with HBV-ACLF exhibited notably diminished levels of testosterone and estrogen at the beginning of the study in comparison to the group without ACLF. As the prevalence of organ failures increased, there was an observed elevation in estradiol levels and a corresponding decrease in testosterone levels. The research findings indicate that testosterone is a more reliable predictor of short-term mortality in individuals with HBV-ACLF during their hospital stay. Additionally, it was observed that estradiol outperformed other predictors in terms of its association with the development of acute-on-chronic liver failure (ACLF). This was shown by an AUROC value of 0.695, which stands for the area under the receiver operating characteristic curve. The study revealed that testosterone could potentially serve as a valuable short-term prognostic indicator for acute-on-chronic liver failure (ACLF) resulting from hepatitis B virus (HBV) infection, while estradiol may contribute to its predictive capabilities.[79]

Yassin et al. (2021) Study: Long-term testosterone therapy improves liver parameters and steatosis in hypogonadal men: a prospective controlled registry study:

The incidence of non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) is commonly observed in Men who don't produce enough testosterone. There is a paucity of research on the enduring impacts of testosterone treatment (TTh) on nonalcoholic fatty liver disease (NAFLD). In 505 hypogonadal males, this study aims to observe and prospectively analyses the cumulative registry data to investigate the liver physiology and function after long-term exposure to testosterone undecanoate (TU). After a preliminary period of 6 weeks, a group consisting of 321 male individuals participated in a treatment plan involving the administration of TU 1000 mg every 12 weeks for a duration of up to 12 years. The group under study will be designated as the T-group, whereas a control group consisting of 184 male individuals made the decision not to undergo testosterone therapy (C-group). The findings demonstrated that in hypogonadal men, long-term TTh treatment improves liver function and hepatic steatosis. Reduced mortality from CVD may have been influenced by improvements in liver function. [80]

Previous research has linked decreased testosterone levels to an increase in body fat percentage. Some scientists propose that adipose **tissue** functions as a complex endocrine gland rather than just a storage site for energy. Inflamed adipose tissue expresses aromatase, which converts testosterone into estradiol, leading to decreased testosterone levels. Elevated levels of TG are a significant risk factor for the development of MetS,

type 2 diabetes, and cardiovascular diseases. Additionally, additional disorders including cancer, bleeding, infection, and TB are impacted by inflammation. Using substances like aromatase inhibitors, which increase testosterone levels while lowering estradiol levels, can therefore lessen inflammation and lower the possibility of developing a variety of pathological illnesses.[81]

In order to examine the relationship between androgen status and inflammatory indicators, the researchers employed testicular-feminized mice in their study, which exhibit reduced levels of endogenous testosterone and inactive androgen receptors, as reported by Kelly et al. According to this study, after 28 weeks, the testicular-feminized mice had considerably higher levels of TNF- and IL-6 than the control group. In the testicular-feminized animals, testosterone therapy (100 mg/mL) only lowered IL-6. The results of this investigation revealed that testosterone had a favorable impact on inflammatory mediators.[82]

Previous studies investigating the relationship between testosterone and molecular markers of inflammation in elderly males have reported that the ageing process is linked to elevated levels of inflammatory cytokines, namely interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-alpha), and interleukin-1beta (IL-1beta). Simultaneously, a relationship can be observed between the phenomenon of ageing and a decline in the level of testosterone (T) in the circulatory system. Numerous empirical studies have provided evidence that the release of testosterone (T) is constrained by the cytokines interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-alpha), and interleukin-1beta (IL-1beta). These cytokines exert their influence on both the central components (hypothalamic-pituitary) and peripheral components (testicular) of the gonadal axis. The use of testosterone supplementation. The available empirical evidence derived from a combination of observational and interventional studies suggests that the utilization of testosterone supplements has been associated with a reduction in inflammatory markers among males with hypogonadism, regardless of their age. The preliminary results were derived from a cohort of 473 elderly males. The CHIANTI research found an inverse connection between T and soluble IL-6 receptor (sIL-6r).[83]

In study conducted in 2005 about Effect of medical castration on CD4+ CD25+T cells, CD8+T cell IFN-gamma expression, and NK cells: a physiological role for testosterone and/or its metabolites.

The data presented by the authors indicates that E2 has the ability to directly increase the expression of IFN- genes in cells that have been activated. Consistent with this observation, a significant reduction in the production of IFN- was observed in activated CD8+ T cells of patients who underwent medical castration and demonstrated decreased serum levels of T and E2. Interferon (IFN) is a cytokine that exhibits significant inflammatory properties it functions as a crucial element of the host's immune reaction to viral infections and the inhibition of tumor proliferation. The study's findings indicate that the reduced activation of CD8+T cells in castrated males may serve as a plausible mechanism by which T and/or its metabolites affect adaptive immunity. The efficacy of the immune response against tumor antigens is largely dependent on the immune response mediated by CD8+T cells. The effectiveness of immune responses against cancer may be impeded as a result of the diminished capacity of CD8+T cells to react to mitogenic stimulation caused by the deprivation of sex steroids. The Natural Killer (NK) cells are known to offer defense against malignancies by exhibiting selective migration towards tumor sites through their primary homing receptor CXCR1. These cells execute tumor cell death through two established mechanisms. MHC class I molecules, which attach to NK cell inhibitory receptors, may be reduced in malignant cells. The activation of the NKG2D stimulatory receptor on natural killer cells is encompassed in the second step [84] ,through the use of ligands specifically designed for tumor targeting. Given the previous demonstration of the predominant involvement of the latter pathway in facilitating the NK cell-mediated antitumor response in male patients afflicted with prostate cancer, the focus of attention is particularly directed towards the expression of NKG2D. The administration of hormone ablation may have an impact on the expression of NKG2D or the homing ability of natural killer cells. The findings of this study indicate that the medical community has yet to fully comprehend the mechanisms through which sex hormones impact the immunological composition and cellular immune function. It is widely accepted that estrogen receptors are present in human peripheral lymphocytes, while the androgen receptor is not. In contrast, immature thymocytes are known to be androgen receptor positive and exhibit clear androgen sensitivity. Nongenomic pathways

may be responsible for transcription-independent signaling that results in the action of androgens on mature lymphocytes, as suggested by study conducted on mice.[85]

A study conducted in 2019 investigated the impact of IL-6 and IL-8 on the activity and function of NK cells, specifically through the STAT3 signalling pathway. The findings of this study demonstrated the essential role of the STAT signalling pathway in the development and maturation of NK cells. The STAT1 protein has the ability to enhance the production of IFN-γ and the cytotoxicity of NK cells. On the other hand, when STAT3 is phosphorylated, it can hinder the immune surveillance of tumours and aid in their evasion from immune regulation. The signalling pathway involving IL-6 plays a role in promoting tumour formation through the activation of STAT3. Despite the increasing of evidence, the effect of sex steroids on the composition of the immune system or the function of cellular immunity is still not fully understood.[86]

Cytokines serve as an important mediator in regulating and coordinating various immune cells involved in the immune response. Proinflammatory cytokines are reduced by testosterone replacement therapy. In the previous investigation in 2006, testosterone replacement therapy reduced IL-1, IL-6, and TNF-γ in antigen-presenting cells in testosterone-deficient males with type 2 diabetes.[87]

Previous research on the functions of androgen/androgen receptor (AR) signaling in hepatocellular carcinoma, fatty liver, cirrhosis, and hepatitis revealed that both normal liver function and the progression of liver pathologies depend on AR signaling.[88] Androgen/AR signalling lowers steatosis, virus-induced hepatitis, and cirrhosis in mice with impaired AR function. Additionally, this research has revealed that modulating AR in mesenchymal stem cells derived from bone marrow (BM-MSCs) enhances their ability to self-renew and migrate, thereby augmenting the therapeutic potential of BM-MSC transplantation for preventing cirrhosis formation.[88] The development of hepatocellular carcinoma (HCC) that is associated with carcinogen or hepatitis B virus has been found to be linked to the signaling of androgen/AR. Nevertheless, Investigations have indicated that the initiation of cancer is primarily attributed to AR as opposed to androgen. Consequently, the pursuit of AR targeting could be deemed a viable therapeutic approach for individuals in the early stages of hepatocellular carcinoma (HCC). On the contrary, it has been evidenced that the signaling of androgen/AR can lead to a reduction in the

metastasis of HCC in patients with a progressed state of the disease. Additionally, there is evidence that Sorafenib combined with medications that boost AR's functional expression may decrease the progression of late-stage HCC.[88] According to the findings of Huang et al. (2013), the presence of androgen/AR had a suppressive impact on the potential of bone marrow-derived mesenchymal stem cells (BM-MSCs) and adipose-derived MSCs to renew themselves. In mice models of liver cirrhosis generated by CCl4 and thioacetamide, it was discovered that this suppression was achieved via the regulation of the EGFR-mediated Erk and Akt pathways. The application of AR-siRNA or ASC-J9, which is an enhancer of androgen receptor (AR) degradation, in bone marrow-derived mesenchymal stem cells (BM-MSCs) resulted in an augmentation of self-renewal and migration capabilities, accompanied by a reduction in inflammation and fibrotic stress. [89] AR-siRNA and ASC-J9 may improve autologous BM-MSC transplantation for cirrhosis. [90]

Chapter Two
Methods

2.1 Experimental design

C57BL/6 male mice at week 12 of age were received care according to the ethical guidelines of An-Najah National University. All animal procedures were approved by the institution's animal care ethics committee (Ref: Med. Oct/2018/59).

Testosterone effects on liver fibrosis

Liver injury mice model were induced using carbon tetrachloride (CCl_4; Sigma, C-5331) introduced by i.p injections of 0.5 µl pure CCl_4/g body weight (one to nine dilution in corn oil) twice a week for two and four weeks as an acute and advanced chronic liver injury. In the middle of the liver injury duration (two week) in the chronic model and one week in the acute model, mice were i.p injected with testosterone (Merck; T1500; purity ≥ 98%) in the concentration of 100 µg/mouse [4 mg/kg mouse body weight] twice a week for the rest weeks. In all experiments, mice were sacrificed two days after the final CCl_4 injection. To this end, the animals were weighed and anesthetized with inhaled 5% isoflurane for 10 seconds before cervical dislocation. Each experimental group included 6 mice.

2.2 Mice groups

The following mice groups were included: (A) Naive mice (mice untreated neither CCl_4 nor testosterone), (B) Mice group treated with testosterone only, (C) CCl_4-treated mice-acute liver injury mode (two-week injections), (D) CCl_4-treated mice-acute liver injury and treated with testosterone, (E) CCl_4-treated mice-chronic liver injury mode (4-week injections), (F) CCl_4-treated mice-chronic liver injury and treated with testosterone.

2.3 Study time and setting

The study was conducted in vivo in an animal mice model.

Setting in vivo study; we need 1-2 months to induce liver injury in mice, then we injected them with testosterone.

2.4 Study variable

Mice

1. Mice Age
2. Mice weight.

In serum analysis

1. Fasting blood sugar
2. Cholesterol
3. Triglyceride
4. Liver enzyme (ALT, AST)
5. IL-6

Real time PCR

1. Alpha smooth muscle actin
2. Collagen

NK cells activity analysis

1. IL-6R
2. INF- γ

2.5 Histological assessment

The liver tissues were subjected to fixation in a 3% formalin solution for a duration of 24 hours, after which an automated tissue processor is used to embed the specimen in paraffin. The sections, measuring 7 μm, were subjected to staining with hematoxylin and eosin (H&E) to check for fatty streaks, inflammatory necrosis, and apoptotic cells. As an additional step, the connective tissue was subjected to staining using a solution of 0.1% Sirius red F3B in a saturated picric acid stain (Abcam, ab150681). Tissue sections were deparaffinized by immersing them in xylene. Sections were then rehydrated by passing them through a series of graded alcohols, starting with absolute alcohol and ending with distilled water. For H&E staining, the sections were stained with hematoxylin, which is a basic dye. They were immersed in a hematoxylin solution for a specific period of time.

Following staining with hematoxylin, the sections were rinsed with running tap water to remove excess stain. Then, the sections were differentiated in an acid alcohol solution to remove excess hematoxylin. The sections were rinsed again with running tap water. The sections were counterstained with eosin, an acidic dye. They were immersed in an eosin solution for a specific period of time. After counterstaining, the sections were dehydrated by passing them through a series of graded alcohols, starting with distilled water and ending with absolute alcohol. Finally, the sections were cleared with xylene and mounted with a coverslip using a mounting medium. For Sirius red F3B staining, the sections were immersed in a Sirius Red solution for a specific period of time to stain collagen fibers. After staining, the sections were washed with distilled water to remove excess stain. The sections were then dehydrated by passing them through a series of graded alcohols, starting with distilled water and ending with absolute alcohol. Finally, the sections were cleared with xylene and mounted with a coverslip using a mounting medium. Veterinary Pathologist assessed all histopathological findings and reported assessments gradings.

For fibrosis area quantification, stained slides were scanned using a Zeiss microscope equipped with image analysis software (ImageJ) through outlining the fibrotic areas within the tissue section. The total fibrosis area was divided by the total number of fields of view or sections examined to get the fibrosis area.

2.6 Liver and metabolic profile assessments in serum

Mouse whole blood samples were obtained on the day of sacrifice and centrifuged at 5000 revolutions per minute for 30 minutes at 4°C. The levels of serum ALT (Abcam; ab285263), AST (Biocompare; MBS2019147), fasting blood sugar (Biocompare; MBS7200879), C-peptide (Biocompare; MBS007738), cholesterol (Abcam; ab285242), and ELISA kits were used to measure cholesterol levels and triglycerides (Biocompare; MBS726589) in line with the instructions provided by the manufacturers. In a nutshell, before using any reagent or sample, they were all warmed to room temperature (about 18 to 25 degrees Celsius). A volume of 100 µL of each standard and sample were added into appropriate wells and incubate for 2.5 hours at room temperature with gentle shaking. The solution was discarded, wells were wash 4 times with 1X Wash Solution, of note; Washing was done by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or auto washer. Following the washing, the liquid was complete removal at each step is essential to good performance. A volume of 100 µL of 1x prepared Detection

Antibody were added to each well for 1 hour at room temperature with gentle shaking. A volume of 100 µL of prepared Streptavidin solution to each well for 45 minutes at room temperature with gentle shaking. A volume of 100 µL of TMB One-Step Substrate Reagent (Item H) were added to each well for 30 minutes at room temperature in the dark with gentle shaking. Finally, 50 µL of Stop Solution (Item I) were added to each well. Absorbance was read at 450 nm immediately using ELISA reader (An-Najah Central Lab).

2.7 C-peptide assessment

Blood samples were obtained from the study participants. The blood samples were collected into appropriate collection tubes or containers. The blood samples were allowed to clot by leaving them undisturbed at room temperature for a specific period of time. After clot formation, the blood samples were centrifuged at a predetermined speed and time to separate the serum or plasma from the clot. The serum or plasma samples were carefully transferred into new tubes, ensuring no contamination or mixing with the clot or red blood cells. The transferred serum or plasma samples were stored at a suitable temperature, such as -80°C, until further analysis, an enzyme-linked immunosorbent test (ELISA) kit (Biocompare; MBS007738) was used to determine plasma levels of C-peptide. Capture antibodies specific to C-peptide were added to each well of the ELISA plates, and the plates were incubated for a set amount of time. After the incubation, the capture antibody solution was removed, and the wells were washed to remove any unbound antibodies. The stored serum or plasma samples were thawed, and appropriate dilutions were made according to the assay kit instructions. The diluted serum or plasma samples were added to the wells of the ELISA plate and incubated for a specific period of time to allow C-peptide binding to the capture antibodies. After the incubation, the wells were washed to remove any unbound materials. Detection antibodies specific to C-peptide were added to each well and incubated for a specific period of time. Following the incubation, the wells were washed to remove any unbound detection antibodies. Substrate solution was added to each well, and the plate was incubated for a specific period of time to allow the substrate to react with the bound detection antibodies. The enzymatic reaction was stopped by adding a stop solution. The absorbance of each well was measured using a microplate reader at a specific wavelength. The C-peptide concentrations in the samples were determined by comparing the absorbance values to a

standard curve generated using known C-peptide concentrations. Data analysis and interpretation were performed based on the obtained C-peptide concentrations.

2.8 RNA isolation, cDNA preparation, and real-time PCR

Triazole buffer (Bio-Lab; Cat# 90102331) extracted hepatic RNA. The liver tissues were homogenized at room temperature, and 0.2 ml of chloroform (Bio Lab; Cat# 03080521) was subsequently introduced. Subsequently, the samples were subjected to incubation at ambient temperature for a duration of 15 minutes, followed by centrifugation at a speed of 1,400 revolutions per minute for a period of 15 minutes at a temperature of 4°C. To perform RNA precipitation, the supernatant from each sample was carefully transferred into a fresh micro-centrifuge tube. Subsequently, 0.5 ml of isopropanol (Bio Lab; Cat# 16260521) was introduced into the tube, and the mixture was incubated at a temperature of 25°C for a duration of 10 minutes. The tubes were subsequently subjected to centrifugation at a speed of 12,000 revolutions per minute for a duration of 10 minutes at a temperature of 4 degrees Celsius. After centrifugation, the liquid portion above the solid pellet, known as the supernatant, was carefully extracted. Following this, one millilitre of a solution consisting of 75% ethanol was introduced to the pellet, and the mixture was subjected to centrifugation at a speed of 7,500 revolutions per minute for a duration of 5 minutes. The pellets underwent air-drying at ambient temperature for a duration of 15 minutes. Subsequently, a volume of 50 µl of diethylpyrocarbonate (DEPC) was introduced to the samples, which were then subjected to a heating process lasting ten minutes at a temperature of 55°C. RNA purification from natural killer (NK) cells was assessed using the RNeasy Plus Mini Kit (catalogue number 74034) in accordance with the guidelines provided by the manufacturer. cDNA was obtained using High-Capacity cDNA Isolation Kit (R&D; Cat# 1406197). RT-PCR reactions were performed using TaqMan Master Mix (Applied Biosystems; Cat# 4371130) to quantify □*SMA*, *collagen III* mRNA levels, Results were normalized to *gapdh* as a housekeeping gene and analyzed using Quant Studio™ 5 Real-Time PCR System.

2.9 ELISA

Serum levels of testosterone and estradiol were assessed using abcam; ab285350 and Creative diagnostics; DEIA04927, respectively. Moreover, intracellular IL-6 and IFN-γ concentrations were assessed using Human IL-6 Quantikine ELISA Kit (R&D; D6050), Human IFN-γ Quantikine ELISA Kit (R&D; 285-IF), according to the manufacture protocols.

2.10 Liver tissue-resident NK (trNK) cells isolation

After liver extraction, Petri plates received 10 ml of DMEM medium (Biological Industries; Cat# 01–055-1A). Homogenised liver tissue was removed and put in 50 ml containers with 10 ml DMEM. After that, the cells were carefully transferred to fresh tubes with Ficoll (Abcam; Cat# AB18115269) and centrifuged at 1600 rpm for 20 minutes at 20°C. Supernatant from each tube was transferred to new tubes and centrifuged at 1600 rpm for 10 minutes at 4°C. After the second centrifugation, each tube's cellular debris was reconstituted in 1 ml of DMEM to separate and purify NK cells. This was achieved using the StemCells kit (Catalog number 19665).

2.11 Flow cytometry

Biological Industries (Cat# 02–023-5A) diluted mouse liver trNK cells to 1 million cells per millilitre in a saline buffer with 1% bovine albumin. Then, the antibodies tagged the cells. Anti-mouse NK1.1 (a marker for murine NK cells) from Biogems (catalogue number 83712-70), anti-CD49a and anti-CD49b from MACS (lot numbers 5150716246 and 5150716256), anti-mouse lysosomal-associated membrane protein-1 (CD107a; a marker for NK1.1 cells cytotoxicity) from eBioscience (catalogue number 48-1071), and anti-IL-6 R from R&D (catalogue number 48-1044). The period is 40 minutes at 4°C incubated the antibodies. Rabbit anti-mice αSMA (R&D; IC1420P) stained pHSCs at 106 cells/mL. The cells were washed with 0.5 ml staining buffer and fixed with 20 ml of 2% paraformaldehyde. A flow cytometer (BD LSR Fortessa™, Becton Dickinson, Immunofluorimetry systems, Mountain View, CA) examined stained cells.

2.12 Statistical analysis

Assessed statistical variances for two-group comparisons, a two-tailed unpaired Student's t-test was used, while for multiple groups, chi-square or one-way ANOVA with Newman-Keuls post-tests was used. GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) was used for statistical analyses. Three repetitions of four sample duplicates were performed.

Chapter Three

Results

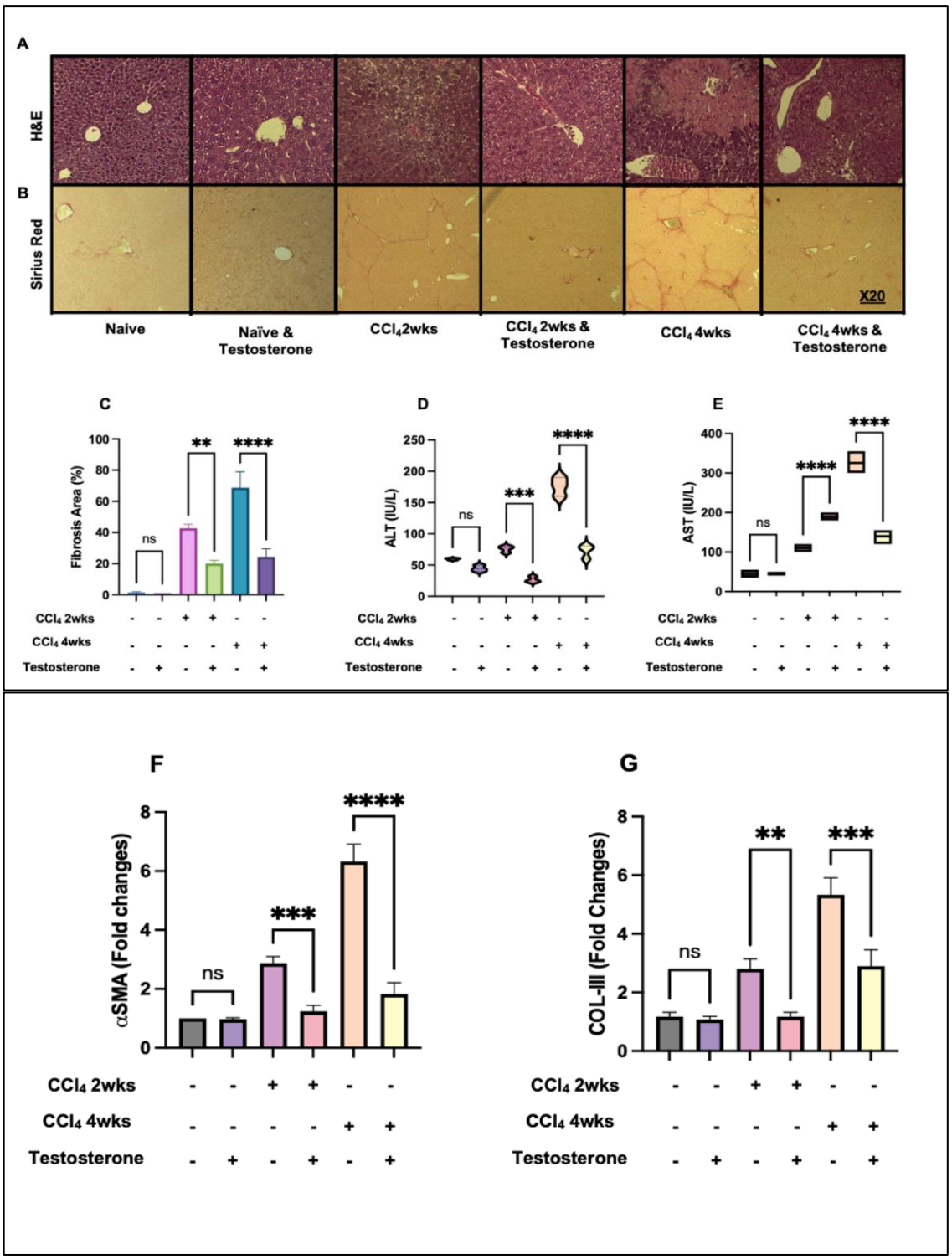
3.1 Characterization of inflammatory and fibrotic profiles in CCl_4 mice treated with testosterone

This research examined how Acute and chronic CCl4 treatments were tested in mice after testosterone delivery for liver damage and phenotypic changes. The present study showcases the hepatic sections of acute and chronic liver fibrosis, which have been subjected to Representative H&E (Figure 7A) and Sirius Red staining (Figure 7B). The histological examination of CCl_4 -induced liver injury using H&E staining revealed the presence of enlarged centrilobular hepatocytes and extensive necrotic regions with significant infiltration of inflammatory cells, accompanied by steatosis. These findings are indicative of the chronic CCl_4 model. Giving a dose of testosterone to mice led to a postponement in the histological outcomes. It was observed that there was a noteworthy decrease in micro- and macrovascular steatosis in the chronic model. The results of Sirius red staining of livers obtained from mice treated with CCl_4 revealed a noticeable rise in collagen deposition in the perisinusoidal regions in both the acute and chronic models of CCl_4. Moreover, the chronic model exhibited a more pronounced effect on collagen deposition. The giving of testosterone led to a significant decrease in the dense fibrous tissue within the stained region when compared to the mice treated with the vehicle. Figure 7C provides a thorough presentation of the histological grading method for H&E and fibrosis examinations.[91][92][93][94] Biochemical markers were evaluated within our sample's cohorts of mice that are under investigation. The study found that there was a linear correlation between the duration of CCl_4 treatments in both the acute and chronic models and the serum inflammatory profiles of ALT (as shown in Figure 7D) and AST (as shown in Figure7E). A 1.32-fold and 1.6-fold improvement was seen after testosterone treatment in the CCl_4 acute model (P0.05). Contrariwise, the chronic CCl_4 model, the giving of testosterone led to a statistically significant reduction of 2.23-fold and 2.1-fold, respectively ($P<0.05$).To validate liver fibrosis in the CCl4-induced animals, we performed quantitative RT-PCR analysis of liver αSMA (Figure 7F) and collagen III (Col III) (Figure 7G) the results obtained from the acute CCl_4-model indicate a significant increase in αSMA and Col III levels by 1.2 and 1.2 folds, respectively $p=0.002$, in mice given an experimental drug as opposed to those treated with a vehicle.

The chronic CCl_4 model exhibited a statistically significant elevation in αSMA and Col III levels, with respective fold increases of 4.2 and 3.2 ($p=0.002$), in comparison to the vehicle-treated mice. Giving of testosterone to mice with liver fibrosis elicited significant reductions in the levels of Col III and αSMA, with reductions of 1.2 and 1.3, respectively ($P<0.03$) observed in the acute CCl_4 - model, and reductions of 2.2 and 2.3, respectively ($P<0.03$) observed in the chronic CCl_4 - model. The results obtained from both RT-PCR and histology evaluations indicate that administration of testosterone treatments led to a reduction in liver fibrosis and an improvement in liver histology with respect to inflammation and fibrosis in liver sections.

Figure 7

The inflammatory and fibrotic profile



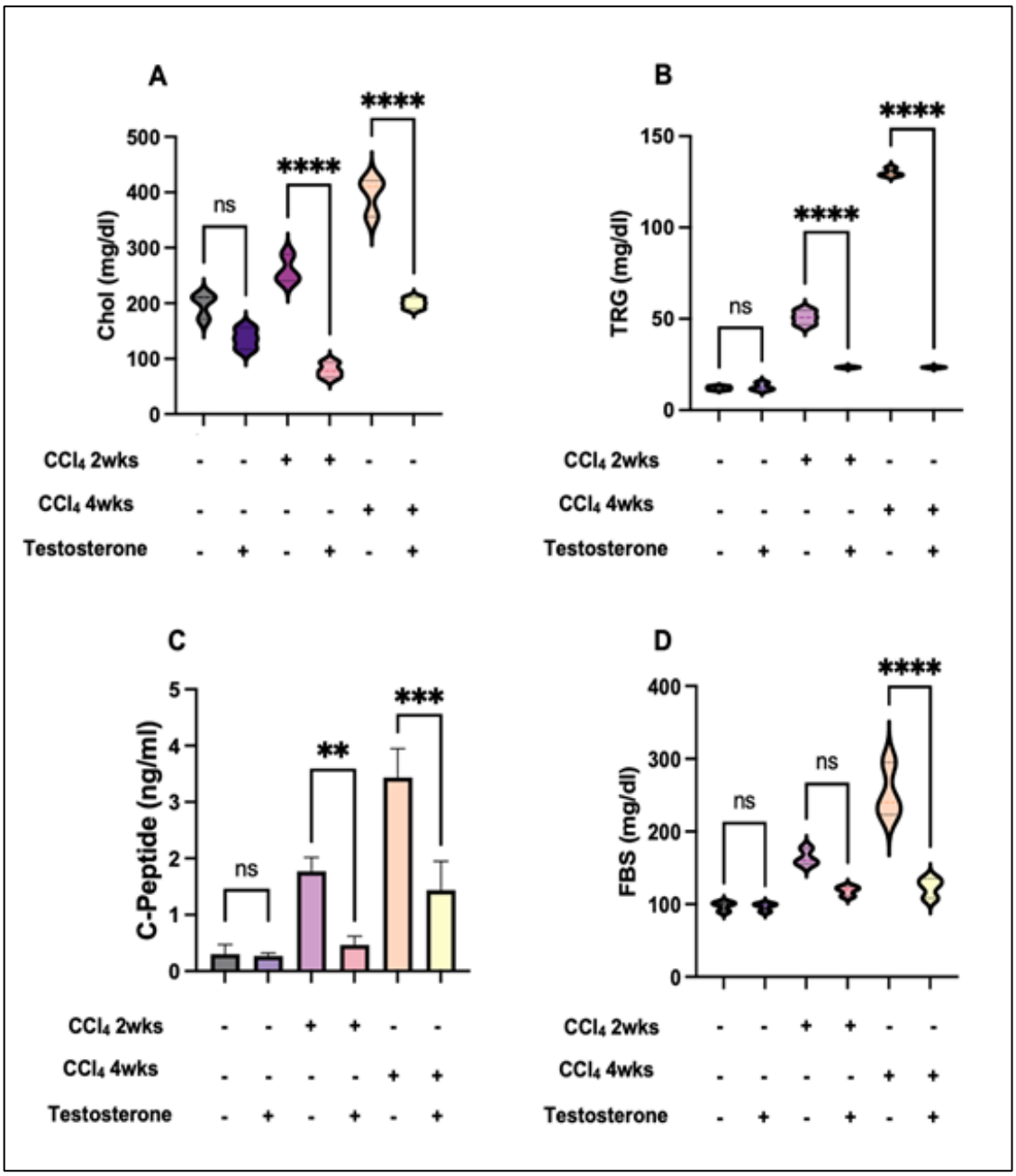
The administration of testosterone has been found to mitigate the histopathological and biochemical manifestations associated with liver fibrosis. Liver injury was experimentally induced in male C57/BL mice for a duration of 2 and 4 weeks and subsequently compared to a control group of naive mice. Testosterone was administered intraperitoneally (i.p.) for a duration of one week and two weeks, commencing at week one and week two of the acute and chronic CCl4 models, respectively, in accordance with the procedures outlined in the Materials and Methods section. The provided figures display immunohistochemical liver staining sections, with Figure A depicting H&E staining and Figure B showcasing Sirius red staining. These images were captured at an initial magnification of 10x. The quantification of liver histology assessments is presented in (C) H&E scoring where than by pathologist according to appendix D as the average ± SEM (Standard Error of Mean) for each group (6 mice per group). Serum markers of liver injury (D) ALT and (E) AST were measured. mRNA indicators of liver fibrosis were (F) αSMA and (G) collagen III. The experiment for each one was repeated three times in order to ensure reliability and repeatability. [**p=0.01, *** p=0.005, ****p=0.0001].

3.2 Metabolic assessments in the CCl_4-induced liver damage mice model

The hepatic alterations in C57BL/6J mice resulting from hypercholesterolemia and induced steatohepatitis are further intensified by the administration of CCl_4. [46] Cholesterol and triglycerides were all shown to be elevated in the livers of rats that were treated with CCl_4 either chronically or acutely. [95] Consequently, the aforementioned model was utilized to delineate metabolic outcome indicators pertaining to lipid and glucose profiles subsequent to interventions involving testosterone. Our CCl_4-induced mice model demonstrated metabolic profile disruption. (Figure 8) shows that serum concentrations of cholesterol (Figure 8A), triglyceride (Figure 8B), C-peptide (insulin) (Figure 8C), and fasting blood sugar (Figure 8D) are all significantly elevated. (Figure 8D) following both acute and chronic treatments of CCl_4 mice. Notably, the chronic treatment model exhibited a more pronounced effect. The liver fibrosis mice that were administered testosterone exhibited sustained low levels of cholesterol, triglycerides, and C-peptide in their serum when compared to the control group that received the vehicle. Additionally, the treated mice displayed a decrease in their fasting blood sugar levels, as depicted in (Figure 8D).

Figure 8

Testosterone improved the perturbed metabolic profile in CCl_4 -induced animals

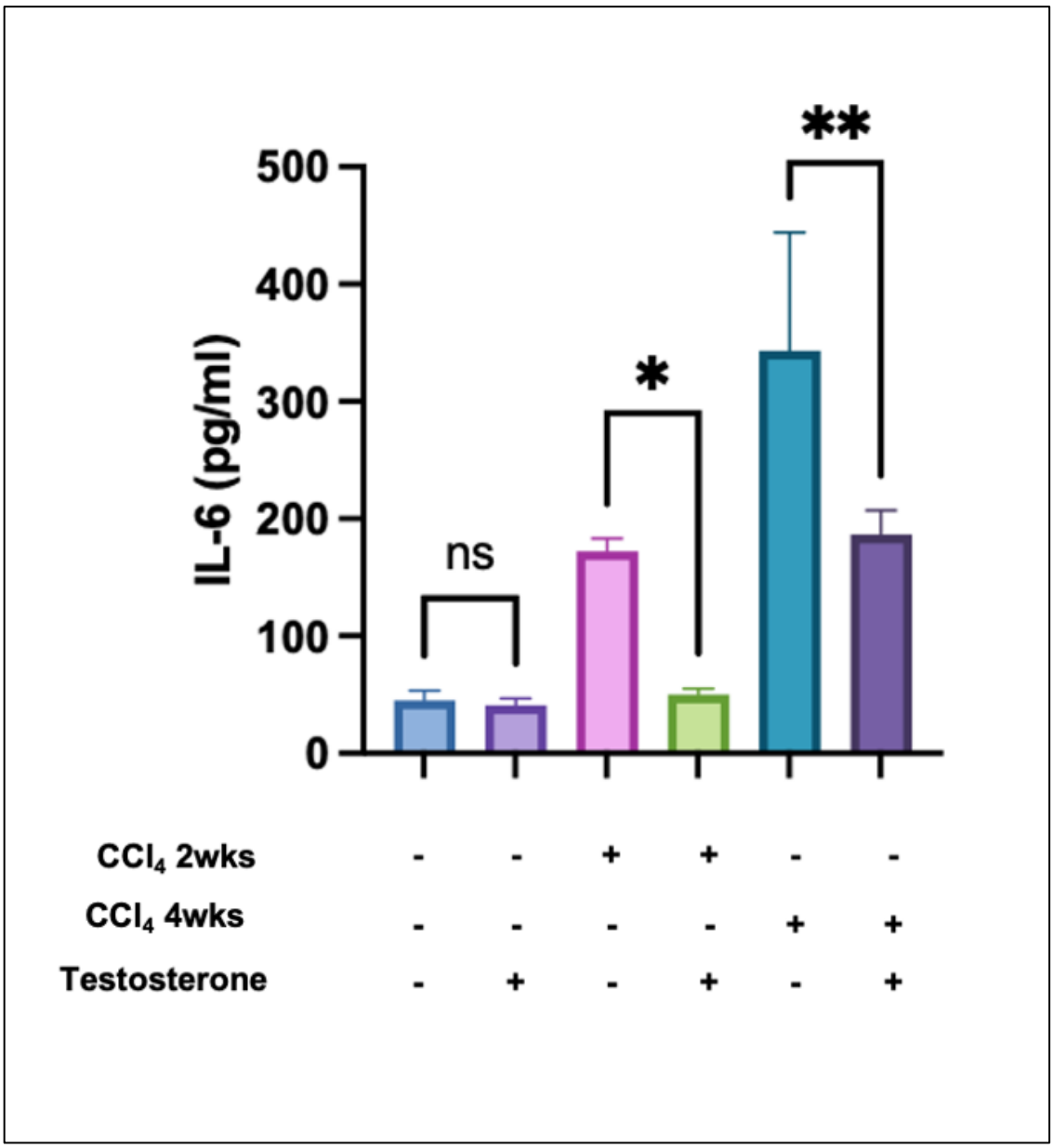


Testosterone improved CCl4-induced metabolic profile in animals. After 16 hours of fasting, the metabolic markers of lipid and glucose profiles in serum levels-cholesterol (CHOL), triglycerides (TRG), C-peptide, and fasting blood sugar (FBS-were examined. The measurements were conducted in triplicate, and the resulting data was presented as the mean value accompanied by the standard error of the mean (SEM).

[*p=0.01, *** p=0.005, ****p=0.0001].

Figure 9

Testosterone displays an inflammatory effect by reducing inflammatory cytokine

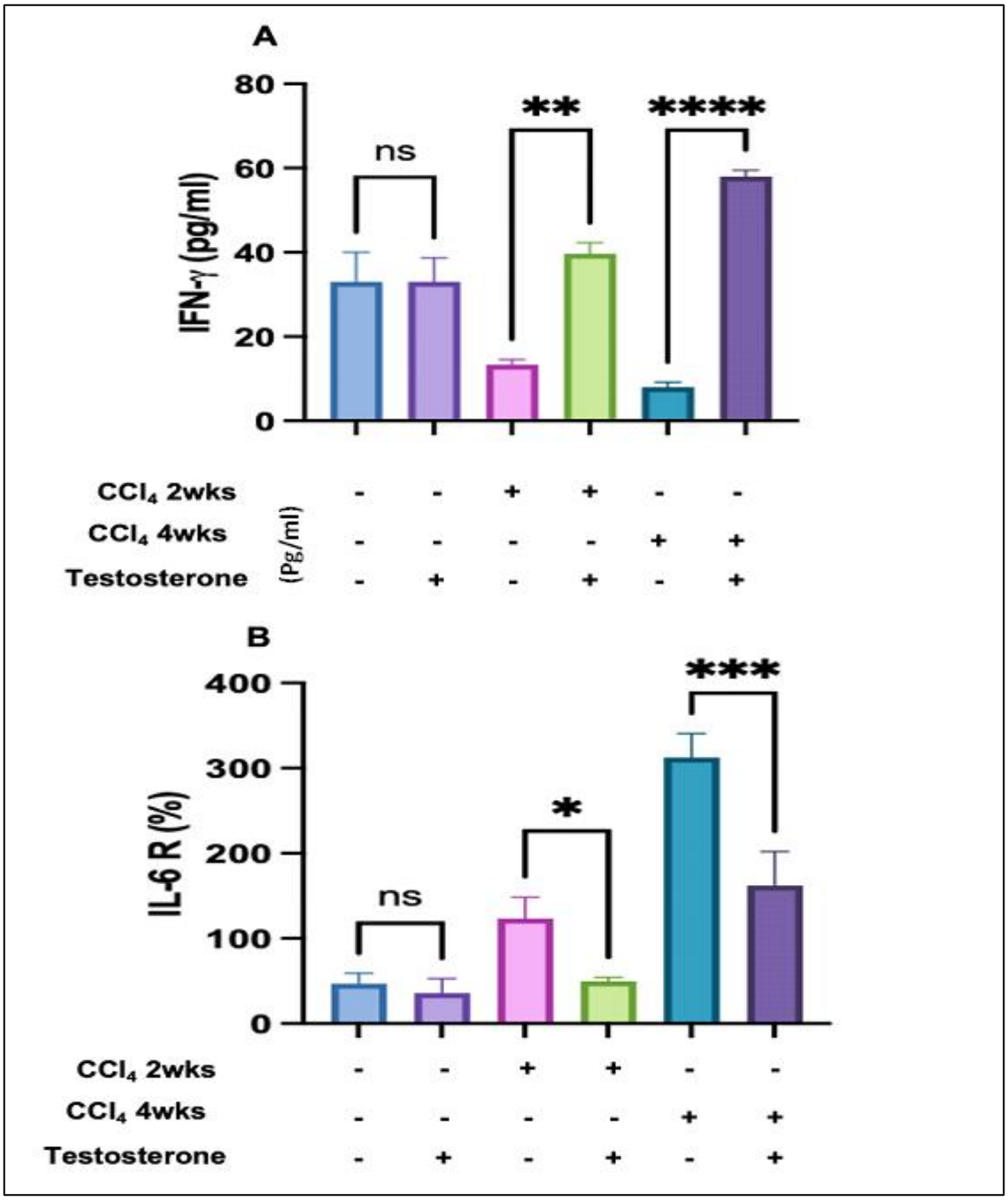


The IL-6 pro-inflammatory cytokine levels were assessed in triplicate for every group. The data underwent analysis through the utilization of a Quantibody Q-Analyzer and a programmed based on Excel. The findings are presented in units of picograms per milliliter (pg/ml). The data of the study is presented in the mean value format, which is accompanied by the standard error of the mean (±SEM). [*p=0.04, ** p=0.012].

3.3 Testosterone treated CCl_4-mice showed liver recruitment of trNK cells and restored their activity

Figure 10

Testosterone ameliorates liver fibrosis by reducing NK INF- γ and improving liver trNK activity



(A) ELISA showed secreted INF- γ in mice in all mice groups. (B) ELISA data demonstrated trNK-IL6 receptor. (Experiments were conducted three times, and results are provided as the mean ± SEM).

Chapter Four
Discussion and Conclusion

4.1 Discussion

This study aimed to determine whether testosterone may mitigate the histological, immunological, and biochemical alterations seen in the liver of CCl_4-mice. Several studies have shown a relationship between testosterone and liver injury.[78][87], even though testosterone treatment improves liver injury and metabolic syndrome and type two diabetes mellitus are well-documented.[35][15] The present comprehension of the influence of testosterone on hepatic injury is limited. The study employed a CCl_4-induced hepatotoxicity model to demonstrate preventative and therapeutic interventions for these agents.[96] Furthermore, prior research has demonstrated that testosterone exerts an anti-inflammatory impact and improves hepatic injury.[19]

The pathogenesis of liver injury is significantly influenced by lipids and glucose, which are also linked to morbidity resulting from atherosclerosis and diabetes. Hepatic stellate cells (HSCs) are activated when free cholesterol is present, and the introduction of cholesterol to a diet that is either high in fat or deficient in methionine/choline results in the buildup of free cholesterol in HSCs. This accumulation of free cholesterol in HSCs expedites the progression of liver fibrosis in experimental models.[97] Excessive accumulation of lipid droplets in hepatocytes is known to exacerbate liver damage by inducing a cascade of pro-inflammatory cytokines, ultimately resulting in steatosis and hepatocyte injury.[98] Prior research has demonstrated that testosterone has Observable impacts on enzymatic pathways associated with the metabolism of fatty acids, regulation of glucose, and utilization of energy. Observed in a consistent manner specific to particular tissues, with varying outcomes observed in diverse regional fat deposits, muscles, and the liver. Such findings may help elucidate the mechanisms underlying the actions of testosterone. The use of testosterone exhibits advantageous outcomes on indices of obesity, which can be attributed to its direct metabolic impact on adipose and muscle tissues, as well as its potential to enhance motivation and energy levels, thereby enabling obese individuals to participate in more physically active routines.[99] After testosterone treatment, cholesterol, triglycerides, C-peptide, and fasting blood sugar levels decreased. The available data suggest that there may be an improvement in liver

histology. Therefore, there is a possibility that the targeting of metabolic profile may serve as a partial remedy for mitigating the advancement of liver fibrosis. The involvement of lipids and glucose as significant risk factors in the development of hepatic injury is noteworthy. Hepatic lipid buildup stimulates liver gluconeogenic peroxisome proliferator-activated receptor (PPAR) ligands, causing hyperglycemia, ketosis, and hyperlipidemia.[100] Hepatic insulin resistance reduces insulin's ability to lower hepatic glucose synthesis, raising blood glucose levels.[101] Support data result present lowering level of cholesterol, LDL, TG and Glucose level after testosterone administration than another group does not treated with it p (Fig .2).

The current study has demonstrated a statistically significant correlation between the testosterone hormone and liver fibrosis by evaluating serum liver injury markers such as ALT and ALT. Aspartate aminotransferase (AST), Alanine transaminase (ALT) indicators of hepatocellular injury. Several studies have demonstrated that high ALT and AST levels are correlated with a higher risk of liver fibrosis, high liver enzymes frequently signify liver cell inflammation or damage. Liver cells that are inflamed or wounded leak more substances into the bloodstream than usual, including liver enzymes, causing liver enzyme levels in the blood to rise.[42][60] A notable reduction in ALT and AST levels was observed in the current study upon the administration of DHT, surpassing the baseline levels of other groups that did not administrate DHT that showed in (Fig.7).

The data presented in our study suggest that administering testosterone can lead to enhanced liver histology. Consequently, it may be possible to mitigate the advancement of liver fibrosis by focusing on the metabolic profile. Real-time PCR analysis was used to examine testosterone's effects on collagen and alpha-smooth muscle expression in a mouse model with liver damage (acute and chronic fibrosis). For instance, collagen and αSMA decrease after testosterone treatment. Previous study reported the severity of human liver fibrosis is associated with increasing αSMA and collagen, the changed ECM composition in progress fibrosis expects that inhibition of ECM degradation is maintained by activated HSCs secreting more amounts of αSMA and collagen. αSMA and collagen levels were significantly higher in chronic fibrosis than acute or absent fibrosis[102], our result that assess decrease in αSMA and collagen after testosterone treatment (Fig. 7).

The immune system is a pivotal component in the mechanism of tissue regeneration. Hence, the regulation of the immune system holds paramount significance in devising the recuperation procedure [38]. The use of EE extract resulted in elevated levels of neutrophil leukocytes and reduced skin fibrosis. However, the mechanism of action for these hepatoprotectives benefit was not described in this research. We analyzed the function of NK cells (trNK) found in isolated liver tissue-resident was separated from different groups of mice and tested. It has been shown that NK cells may have an antifibrotic impact by eliminating activated HSCs. [103]. As can be shown in Figure 10A, there is a 2-fold-decrease ($p=0.003$) in the degree of liver fibrosis in conjunction with trNK INF-γ secretions. Following testosterone treatment, trNK secretions of INF-γ showed higher levels of 2.1 and 6.3 folds in the acute and chronic model, respectively. To further associate trNK activatory effects following testosterone treatment with IL-6 receptor, The ELISA procedure was conducted in accordance with the instructions outlined in the Materials and Methods section. Figure 10B shows that IL-6 receptor levels decreased 1.6-fold and 2.5-fold in acute and chronic liver fibrosis models, respectively, in the experimental group of mice compared to the control group that received the vehicle ($p=0.0001$).

The results of our study offer several indications regarding the potential advantages of testosterone therapy in ameliorating liver fibrosis and improving liver histology through the reduction of inflammation and fibrosis in liver sections. These data accomplished were mediated through a decrease in NK IL-6 receptors and consequently restored NK activity. The findings suggest that testosterone possesses anti-inflammatory and anti-fibrotic properties and underscore its potential as an immunological target for the purpose of retarding liver fibrosis.

In summary, the data presented indicate that testosterone possesses antifibrotic properties, which can be attributed to its capacity to improve lipid and glucose profiles. These factors are acknowledged as risk factors in the advancement of fibrosis. The results of our investigation indicate that testosterone could potentially be utilized as a feasible therapeutic intervention to delay and hinder liver fibrosis by augmenting insulin sensitivity. To delve deeper into the underlying mechanism of testosterone's antifibrotic effects, an additional evaluation was conducted to examine the potential contribution of inflammatory and immune factors in mitigating liver fibrosis. The study evaluated the

concentrations of Serum IL-6, the functionality of isolated liver tissue-resident NK (trNK) cells, and the levels of trNK IL-6 receptors. Testosterone has been shown to have immune-modulating properties. In vitro, studies have indicated that testosterone may have the ability to inhibit the expression of proinflammatory cytokines such as TNFα, IL-1β, and IL-6 while enhancing the expression of the anti-inflammatory cytokine IL-10. [104][82] In addition, the study revealed that testosterone has anti-inflammatory properties. Furthermore, it demonstrated a significant inhibitory effect on the growth of adipose tissue and the expression of various adipocytokines, such as leptin, TNF-α, IL-6, and IL-1, while exhibiting a positive correlation with adiponectin level. Conversely, a diminished level of testosterone was associated with an elevated expression of inflammation markers. In our research study, presented in Figure 9 displayed both naïve mice treated and untreated with testosterone had comparable low levels of serum IL-6 of 65±10 pg/ml (p=ns). Serum IL6 levels were linearly correlated with liver fibrosis severities of 180±24 pg/ml and 345±52 pg/ml in the acute and chronic models, respectively (p=0.002). There was a 2.4-fold (p0.0001) and 2.3-fold (p0.0003) reduction in testosterone levels after testosterone therapy in the acute and chronic settings, respectively (p0.002). The anti-inflammatory effect of testosterone is attributed to the decrease in inflammatory cytokines.[38]

4.2 limitation

Although this research has met its aim, and the research methodology was carefully designed, due to time and budget constraints this study has several important limitations. The studies about a relationship between liver fibrosis and testosterone is very limited, the time was limited in our study to estimate more parameter such estradiol and androgen receptor , our experiment done on mice model that become more aggressive after inject CCL4 so very difficult to deal with it.

4.3 Conclusion

Testosterone has anti-inflammatory and anti-fibrotic effect by storing histology for decreasing in the levels of αSMA and Col III and decreasing of ALT and AST level. Testosterone improved metabolic profile in CCl4 -induced animals by decrease cholesterol, triglyceride, FBS and C-peptide level. Testosterone exhibits an inflammatory response by diminishing the levels of inflammatory cytokine, specifically leading to a

decrease in IL-6. Testosterone treated CCl4-mice showed trNK cells restored their activity, by increase NK INF-g and a decrease in IL-6 receptor levels. These results highlight the immune-modulatory effects of testosterone, which are associated with its anti-inflammatory and anti-fibrotic properties. This suggests that testosterone may serve as a valuable approach in the treatment of liver conditions characterized by inflammation and fibrosis.

It can be inferred from our findings that testosterone exhibits therapeutic promise in the amelioration of liver fibrosis, as evidenced by its positive impact on the histological characteristics of inflammation and fibrosis within hepatic tissue. This improvement was caused by a reduction in NK IL-6 receptors, which in turn led to a restored their NK activity, emphasizing the immune modulatory effects of testosterone associated with its anti-inflammatory and anti-fibrotic properties.

List of abbreviations

Abbreviation	Meaning
ACLD	Advanced chronic liver disease
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
AR	Androgen receptor
αSMA	Alpha smooth muscle actin
CCl_4	Tetra-Carbone chloride
Chol	Cholesterol
CRP	C-reactive protein
DHT	Dihydrotestosterone
DMEM	Dulbecco's Modified Eagle's Medium
ECM	Extra cellular matrix
FSH	Follicle stimulating hormone
Glu	Glucose
GnRH	Gonadotropin-releasing hormone.
GSc	Gleason score
HDL	High-density lipoprotein
HSCs	Hepatic stellate cells
IL-6	Interleukin 6
IL-6R	Interleukin 6 receptor
LH	Luteinizing hormone
NK	Natural killer cell
PPAR	Peroxisome proliferator-activated receptor
PCOS	Polycystic ovarian syndrome
PBC	Primary biliary cirrhosis
ROS	Reactive oxygen species
RT-PCR	Real-time polymerase chain reaction
SEM	Standard Error of Mean
SRY	Sex-related gene on the Y chromosome
SPSS	Statistical package for social science
TTh	Testosterone treatment
trNK	Tissue-resident NK cells
T2D	Type II diabetes mellitus
TG	Triglyceride

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Appendices

Appendix A

Approval from Faculty of Graduate Studies

نموذج تحديد عنوان الأطروحة و المشرف

*** يجب توفر جميع الشروط التالية لتحديد عنوان الأطروحة و المشرف :
- أن يكون مسار الطالب أطروحة ** الشرط متحقق **
- أن يتم الطالب 12 ساعة . ** الشرط متحقق ** ** عدد الفصول أكبر من 4 **
- أن لا يكون الوضع الدراسي للطالب "مفصول من البرنامج". ** الشرط متحقق **
- المعدل التراكمي للطالب أكبر أو يساوي من 2.8 ** الشرط متحقق **

اسم الطالب :	هديل منير عادل صنوبر	رقم التسجيل :	11952474
اسم البرنامج :	ماجستير الكيمياء الحيوية السريرية	مسار الدراسة:	أطروحة
عدد الساعات المعتمدة التي انجزت حتى الان:	27	المعدل التراكمي:	3.21
الوضع الدراسي :	يدرس		
عنوان الطالب :	فريوت خانيلس	رقم الهاتف المحمول :	0597786252
البريد الالكتروني :	hadeelsnober3@gmail.com		
لغة الرسالة :	انجليزي		
عنوان الأطروحة باللغة العربية :	تأثير تليف الكبد على التسبب في سرطان البروستاتا		
عنوان الأطروحة باللغة الانجليزية:	The impact of liver fibrosis on the pathogenesis of prostate cancer		
النسخة الإلكترونية من مقترح الاطروحة :	docx.11952474-1		

رقم المشرف الأول :	3586	اسم المشرف الأول:	جوني يعقوب نصري عامر
المشرف الثاني :	يعمل في جامعة النجاح: ----------		

ملاحظة المشرف :	na			التاريخ :	2022-02-02
ملاحظة المنسق :	موافق	رأي المنسق :	موافق	التاريخ :	2022-02-07
ملاحظة رئيس القسم :	موافق	رأي رئيس القسم :	موافق	التاريخ :	2022-02-07
ملاحظة مدقق الدراسات :	يعرض على مجلس الكلية	رأي مدقق الدراسات :	موافق / عبء الاشراف : أدنى من الحد	التاريخ :	2022-02-07
ملاحظة عميد الدراسات العليا :	لامانع جلسة 415	رأي عميد الدراسات العليا:	موافق	التاريخ :	2022-02-07

قرار مجلس الكلية			
تم تغيير العنوان من قبل مجلس الكلية :	لا		
عنوان الأطروحة باللغة العربية :	تأثير تليف الكبد على التسبب في سرطان البروستاتا		
عنوان الأطروحة باللغة الانجليزية:	THE IMPACT OF LIVER FIBROSIS ON THE PATHOGENESIS OF PROSTATE CANCER		
رقم المشرف:	3586	اسم المشرف:	جوني يعقوب نصري عامر
المشرف الثاني :	يعمل في جامعة النجاح: ----------		
فصل الاعتماد :	الثاني	سنة الاعتماد :	2021 ** ملاحظة : مثال العام الدراسي 2021-2022 يتم ادخاله على شكل 2021
رقم جلسة الكلية:	415		
تاريخ جلسة الكلية:	3/2/2022		

Appendix B

نموذج تعديل عنوان الأطروحة قبل المناقشة

Reload Page

An-Najah National University
Faculty of Graduate Studies
Dean's Office

جامعة النجاح الوطنية
كلية الدراسات العليا
مكتب العميد

نموذج تعديل عنوان الأطروحة قبل المناقشة

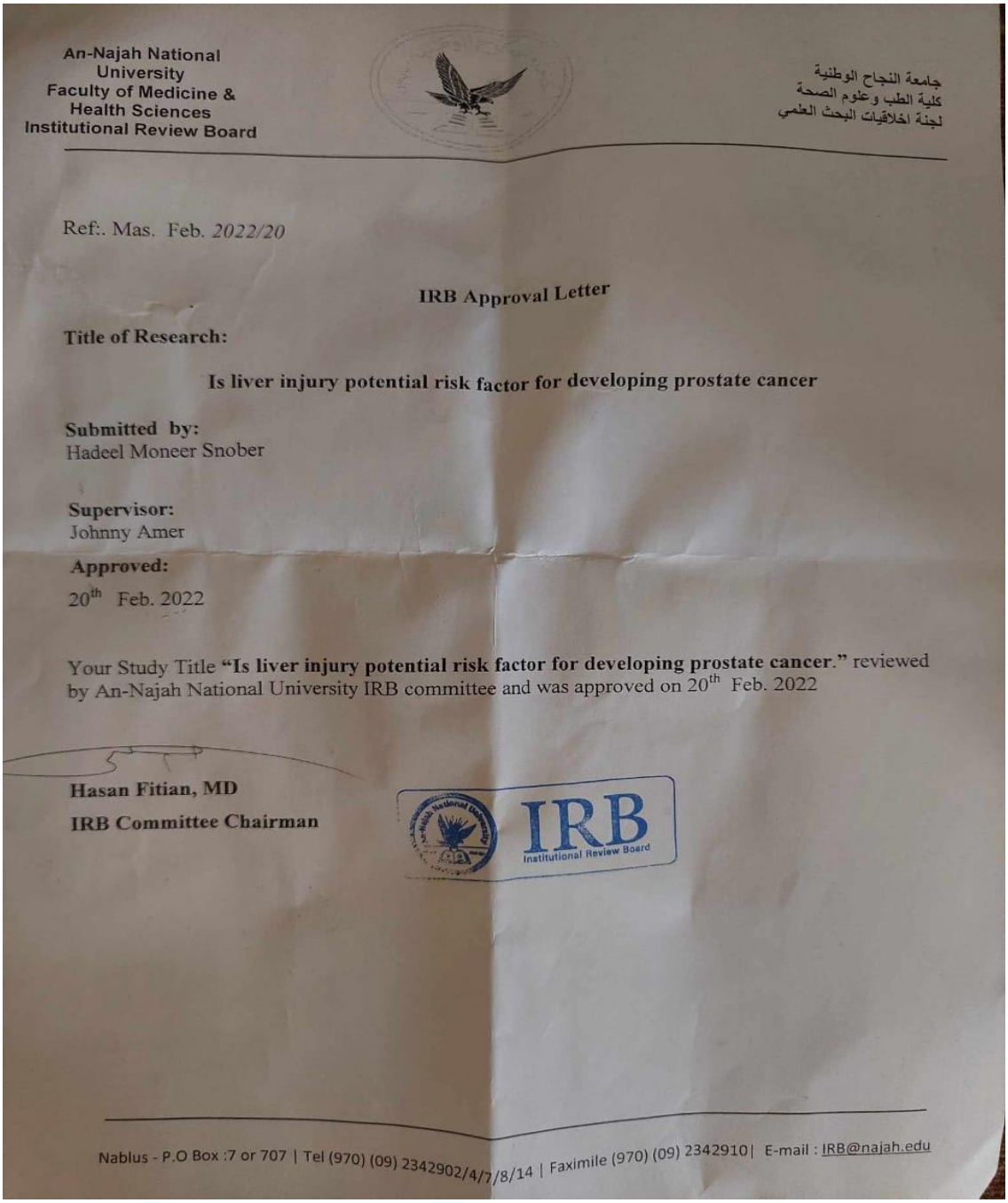
***** يجب توفر جميع الشروط التالية :**
- أن لا يكون الوضع الدراسي للطالب "مفصول من البرنامج". ** الشرط متحقق **
- ان يكون مسار الطالب اطروحة. ** الشرط متحقق **
- أن لا يكون الطالب قدم نموذج تشكيل لجنة مناقشة. ** الشرط متحقق **

التاريخ : 10-04-2023			
رقم الطالب :	11952474	اسم الطالب :	هديل منير عادل صنوبر
اسم البرنامج:	ماجستير الكيمياء الحيوية السريرية	مسار الدراسة:	أطروحة
عدد الساعات المعتمدة التي انجزت حتى الان:	30	عدد الساعات المعتمدة :	0
المعدل التراكمي:	3.27	رقم الجوال:	0597786252
عدد الفصول الدراسية :	7		
البريد الالكتروني:	hadeelsnober04@gmail.com	عنوان الطالب :	قريوت نابلس
لغة الرسالة :	انجليزي		
عنوان الرسالة القديم باللغة العربية :	تأثير تليف الكبد على التسبب في سرطان البروستاتا		
عنوان الرسالة القديم باللغة الانجليزية :	THE IMPACT OF LIVER FIBROSIS ON THE PATHOGENESIS OF PROSTATE CANCER		
مشرف أول | 3586 | جوني يعقوب نصري عامر			
مبررات التعديل على العنوان:	in the practical part we inject CCL4 at the beginning and later on we inject testosterone -1 so we asses the effect of testosterone in liver injury in mice model		
	few article in this field and unclear role of testosterone effect in liver injury -2		
عنوان الأطروحة الجديد باللغة العربية:	التأثير المناعي والايضي لهرمون التستوستيرون على تليف الكبد لنموذج الفئران		
عنوان الأطروحة الجديد باللغة الانجليزية:	The immune and metabolic impact of testosterone on mice model of liver fibrosis		

ملاحظة المشرف :	The immune and metabolic impact of testosterone on mice model of liver fibrosis			
رأي عميد الدراسات العليا:	موافق	ملاحظة :	لا مانع - جلسة 428	التاريخ : 2023-04-12
				التاريخ : 2023-04-12

Appendix C

IRB Approval



An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref:. Mas. Feb. *2022/20*

IRB Approval Letter

Title of Research:

Is liver injury potential risk factor for developing prostate cancer

Submitted by:
Hadeel Moneer Snober

Supervisor:
Johnny Amer

Approved:
20th Feb. 2022

Your Study Title **"Is liver injury potential risk factor for developing prostate cancer."** reviewed by An-Najah National University IRB committee and was approved on 20th Feb. 2022

Hasan Fitian, MD
IRB Committee Chairman

IRB Institutional Review Board

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

Appendix D

Liver histological evaluation for fatty degeneration and fibrosis; Histological Assessment

The following parameters were evaluated (H&E):

Item	Definition	Score/Code
Steatosis		
Grade	Low- to medium-power evaluation of parenchymal involvement by steatosis	
	<5%	0
	5%-33%	1
	>33%-66%	2
	>66%	3
Location	Predominant distribution pattern	
	Zone 3	0
	Zone 1	1
	Azonal	2
	Panacinar	3
Microvesicular steatosis*	Contiguous patches	
	Not present	0
	Present	1
Fibrosis		
Stage		
	None	0
	Perisinusoidal or periportal	1
	Mild, zone 3, perisinusoidal	1A
	Moderate, zone 3, perisinusoidal	1B
	Portal/periportal	1C
	Perisinusoidal and portal/periportal	2
	Bridging fibrosis	3
	Cirrhosis	4
Inflammation		
Lobular inflammation	Overall assessment of all inflammatory foci	
	No foci	0
	<2 foci per 200× field	1
	2-4 foci per 200× field	2
	>4 foci per 200× field	3
Microgranulomas	Small aggregates of macrophages	
	Absent	0
	Present	1
Large lipogranulomas	Usually in portal areas or adjacent to central veins	
	Absent	0
	Present	1
Portal inflammation	Assessed from low magnification	
	None to minimal	0
	Greater than minimal	1
Liver cell injury		
Ballooning*		
	None	0
	Few balloon cells	1
	Many cells/prominent ballooning	2
Acidophil bodies		
	None to rare†	0
	Many	1
Pigmented macrophages		
	None to rare†	0
	Many	1
Megamitochondria*		
	None to rare†	0
	Many	1

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

التأثير المناعي والايضي لهرمون التستوستيرون على تليف الكبد لنموذج الفئران

إعداد

هديل صنوبر

إشراف

د. جوني عامر

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

2023

التأثير المناعي والايضي لهرمون التستوستيرون على تليف الكبد لنموذج الفئران

إعداد

هديل صنوبر

إشراف

د. جوني عامر

الملخص

الخلفية: أظهرت الخلايا القاتلة الطبيعية (NK) تأثيرًا مضادًا للتليف الكبدي. ومع ذلك، يُعتقد أن وظيفتها تتعطل في إصابة الكبد المتقدمة. في الدراسة الحالية، هدف الدراسة إلى تقييم التأثير المناعي والتمثيل الايضي لهرمون التستوستيرون على نماذج الفئران المصابة بالتليف الكبدي الحاد والمزمن.

المنهجية: تم إجراء حقن لرابع كلوريد الكربون لمدة (أسبوعين) لاحداث الاصابة بالتليف الكبدي الحاد و لمدة (4 أسابيع) لأحداث التليف الكبدي المزمن عند ذكور الفئران. تم حقن التستوستيرون (4 مجم / كجم من وزن الفأر) بطريقة الحقن داخل الصفاق (Intraperitoneal Injection) بعد الأسبوع الأول من حقن النموذج الحاد لرابع كلوريد الكربون وبعد الأسبوع الثاني من حقن النموذج المزمن لرابع كلوريد الكربون ، تم تشريح الفئران ، وتم جمع المصل لتقييم مستويات إنزيمات الكبد (AST, ALT) وعلامة الالتهاب (IL-6)، والببتيد (C) وكذلك الدهون والجلوكوز. تم أخذ الكبد واستخدامه في التقييمات النسيجية للالتهابات والتليف. للدلالة على درجة التليف الكبدي؛ تم تقييم (αSMA) و (Collagen III) بواسطة (RT-PCR) علاوة على ذلك، تم عزل خلايا (NK) المقيمة في أنسجة الكبد وتقييم نشاطها من خلال تقييم مستقبلات (INF) و(IL-6) بواسطة (ELISA) وقياس التدفق الخلوي على التوالي.

النتائج: تم تقييم مستويات انزيمات الكبد (ALT) و(AST) و(IL-6) والتقييمات الأيضية للكوليسترول والدهون الثلاثية والببتيد (C) وسكر الدم الصائم ,حيث اظهرت النتائج علاقة خطية بين المؤشرات وتطور التليف. وتم تقييم المظهر النسيجي للكبد فكان يظهر أسوأ في نموذج التليف المزمن لإصابة الكبد. كمان

اظهرت نتائج الفئران المعالجة بالتستوستيرون انخفاضًا كبيرًا في ترسب الكولاجين في أنسجة الكبد. علاوة على ذلك، أظهرت علاجات التستوستيرون انخفاضًا كبيرًا في مصل (IL-6) بمقدار 2.4 ضعفًا و2.3 ضعفًا في النموذجين الحاد والمزمن على التوالي وارتبطت البيانات بزيادة في إطلاق الانترفيرون جاما من الخلايا الطبيعية القاتلة دلالة على دور التستوستيرون في استعادة نشاط خلايا الطبيعية القاتلة.

الخلاصة: أظهرت نتائجنا تأثيرات هرمون التستوستيرون في انخفاض مستويات مستقبلات (NK IL-6) وبالتالي تسبب في تنشيط الخلايا الطبيعية القاتلة ، والنتائج التي يمكن أن تفسر جزئيًا التحسن في إصابة التليف بالكبد. كما تشير نتائجنا إلى ان هرمون التستوستيرون اظهر دوره كعلاج مضاد للالتهابات ومضاد للتليف وتأخير تطور التليف الكبدي.

الكلمات المفتاحية: التأثير المناعي والايضي؛ التستوستيرون؛ تليف الكبد؛ نموذج الفئران.